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Differences in egg thermotolerance between tropical and temperate populations of the migratory locust *Locusta migratoria* (Orthoptera: Acridiidae)

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Abstract

The migratory locust *Locusta migratoria* L., which is widely distributed throughout the world, exhibits within- and between-population variation in cold tolerance. To understand physiological adaptation in populations, we studied the genetic basis of thermotolerance in Hainan (tropical) and Liaoning (temperate) populations and measured expression of *Hsp*70 and *Hsp*90 mRNA in both populations at low (0 °C) and high temperatures (40 °C). Phenotypic variation of thermotolerance is heritable. Heritable characteristics differed among different stages of locust egg development, as well as among different measures of thermotolerance. Nuclear genetic factors, rather than cytoplasmic factors, contribute to differences in cold tolerance between the tropical and temperate populations of the migratory locust; for heat tolerance, maternal effects were involved in three stages of egg development. Expression of *Hsp*90 mRNA was induced in temperate population after heat shock (40 °C × 12 h), whereas expression of Hsp70 and 90 was induced in tropical population after cold shock (0 °C × 12 h). We suggest that thermotolerance of locust eggs has a complex genetic basis and heat shock proteins may be involved in differences of thermotolerance between locust populations.

Keywords: Genetic variation; Heat shock proteins; Inheritance; *Locusta migratoria*; Thermal stress

1. Introduction

For ectotherm species, such as insects, temperature has long been recognized as a major environmental factor responsible for species abundance and geographic distribution (Leather et al., 1993). Thus, the capacity to adapt to and tolerate extreme temperature is critical for the persistence of populations. When exposed to extreme temperatures, insects may respond in different ways: they could behaviorally avoid extremes by escaping the adverse conditions, or respond through changes in morphology, life history and/or physiology (Hoffmann and Parsons, 1991).

The migratory locust *Locusta migratoria* L., which is widely distributed throughout the world (Ma, 1962), has exhibited within- and between-population variation in cold tolerance (Jing and Kang, 2003, 2004). In China, northern populations of locusts were more cold hardy than southern populations. Populations that develop in autumn have a significantly lower supercooling point (SCP) and lethal temperature than those developing in summer and post-winter. In addition, first instar hoppers of the migratory locust exhibit a rapid cold hardening response that protects against large temperature fluctuations in spring or early summer (Wang and Kang, 2003). Although heat shock proteins (Hsps) can also be expressed at a high level by thermal stress in migratory locust (Whyard et al., 1986; Baldaia et al., 1987) and may function as an adaptation to

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the locust's chronic exposure to heat shock temperatures (Qin et al., 2003), the relationship between expression of Hsps and thermotolerance of natural populations of migratory locust remains unknown.

Hsps are families of proteins (Hsp100, 90, 70, 60, and 20) that are known to assist in the folding and translocation of newly synthesized proteins, as well as in coping with stress-induced denaturation of other proteins (Nover and Scharf, 1997). A strong correlation has been found between Hsp70 expression and thermotolerance in diverse species (Feder and Hofmann, 1999). For instance, Hsp70 improves thermotolerance in both embryos (Welte et al., 1993) and larvae (Feder, 1996) of *Drosophila melanogaster*, and natural variation of Hsp70 expression in larvae correlates with thermotolerance (Feder and Krebs, 1997). Hsp90 differs from other Hsps in that it is constitutively expressed and has a dual role in folding of non-native proteins (Wiech et al., 1992), peptide translocation (Brugge et al., 1981) and signal transduction (Pandey et al., 2000). Furthermore, effects of Hsp90 on the buffering and release of genetic variation suggests that they may have an impact on evolutionary processes (Queitsch et al., 2002).

A specific problem in thermal adaptation investigations arises from the fact that the roles of genetic background and random factors may be large in evolution and cannot be easily considered by comparing populations for different traits (Travisano et al., 1995; Chown, 2001). Gene flow between populations can cause an artificial appearance of a correlation between a phenotype and an environmental variable (Beerli and Felsenstein, 1999, 2001; Felsenstein, 2002). Growth temperature variations can also induce a diversity of thermotolerance (Ayrinhac et al., 2004). Therefore, although we had found clinal variation of cold hardiness with latitude in the migratory locust (Jing and Kang, 2003), population comparisons have been unable to distinguish between changes in cold hardiness that are due to phenotypic plasticity (acclimation) and those that have an adaptive basis (Huey and Berrigan, 1996).

Here, using genetic crossing experiments and a molecular expression analysis for two extreme populations, we aim to address the following questions: (1) Does cold tolerance have a genetic component at population levels? (2) If heritable, what are the genetic factors that control cold and heat tolerance in different developmental stages? (3) Is genetic variation in thermotolerance between populations associated with expression of Hsps?

2. Materials and methods

2.1. Insects

Individuals of L. *migratoria* were collected in June 2002 from two extreme sites of the species range in China: Hainan Province (18°23′N, 109°30′E) in the tropical region and Liaoning Province (41°10′N, 122°06′E) in the temperate region. In Hainan Province, the mean monthly temperature is 18 °C in winter and 29 °C in summer; whereas in Liaoning Province, the mean monthly temperature is −10 °C in winter and 21 °C in summer (Liu, 1995). The population from Hainan Province was designated as H and the population from Liaoning Province as L.

Fifth instar hoppers of migratory locusts were brought back to the laboratory. All insects were reared in two-floor boxes (50 × 70 × 80 cm) with wheat bran and wheat seedlings at 30 °C (14 h photoperiod). Sand was used as the oviposition medium. Newly laid eggs were collected daily. The egg pods were incubated at 30 °C in the dark, and individual eggs were separated carefully from the egg pods using a brush.

2.2. The crossing experiment and test of laboratory effect

The parental and reciprocal crosses of the migratory locust (i.e. male (♂) H × female (♀) H, ♂ L × ♀ L, ♂ H × ♀ L, ♂ L × ♀ H) were established from H and L populations collected directly in the field. Both crosses produced fertile males and females. Hybrid males were backcrossed with females of the parental stock. In total, three replicates with 50 pairs in each cage were set up for each cross and each generation.

In addition, because the difference between natural environment and rearing environment in the laboratory may affect thermotolerance of locust eggs, we compared thermotolerance of the F1 generation with that of the parental generation.

2.3. Assessment of thermotolerance

During the life cycle of the migratory locust, the immobile egg stage is more prone to thermal stress than other stages because the locust eggs are in a quiescence stage. Based on morphological characteristics, embryonic development was divided into three stages: anatrepsis (stage I), balstokinesis (stage II), and katatrepsis (stage III). In this study, we selected 2-, 7- and 12-day-old eggs incubated at 30 °C to represent stages I–III, respectively (Uvarov, 1966). Because cold tolerance of the locust eggs exhibited variations among the different stages of development and were associated with seasonal adaptation, we estimated heritable characteristics of thermotolerance in the three developmental stages. Thermotolerance was examined at three developmental stages of two parental stocks and F1 hybrids of reciprocal crosses. Thermotolerance of the backcross progeny with F1 hybrids and the parental stocks were only measured at stage II because diapause may exist in stage II for overwintering in the northern population.

To evaluate survival at low temperature, groups of 40 eggs from each stock, incubated at 30 °C for 2, 7 or 12 days were confined in glass tubes sealed with parafilm and exposed to −5 °C for different durations (6 h, 1, 3, 5 and 10 days). Then, the tubes were taken out, the eggs were removed and transferred into a 100 ml plastic box with moist sand (15% water content) and filter paper, and allowed to recover in the laboratory at 30 °C. Survival was measured based on the number of eggs that hatched. As a control experiment, 40 eggs were put directly into a plastic box and kept at 30 °C. This process was repeated four times.

To test for heat tolerance, groups of 40 eggs from each stock at 30 °C for 2, 7, and 12 days were also confined in glass tubes sealed with parafilm and immersed in a water bath maintained at 42 ± 0.1 °C for varying periods of time (12 h, 1, 2, 3, and 5 days). Other procedures were similar to those for measurement of cold tolerance.

The cold and heat tolerance of the locust eggs were assessed in terms of "lethal time$_{50}$" (LT_{50}), which was defined as the time taken to achieve 50% mortality at a given treatment temperature. We initiated LT_{50} measurements by holding locust eggs at two temperatures that we expected to lead to 0% and 100% mortality (0 and −5 °C, respectively). We repeated these assays with intermediate durations until an LT_{50} was reached (Jing and Kang, 2003).

2.4. mRNA expression of Hsp70 and Hsp 90

2.4.1. Temperature treatment and total RNA isolation

Seven-day-old locust eggs of the two parental populations (H and L) were frozen with liquid nitrogen after cold shock (0 °C × 12 h) and heat shock (40 °C × 12 h). Eggs incubated at 30 °C were used as control. Total RNA from the three treatments was isolated using a commercial kit (Rneasy® Mini kit (50), QIAGEN Corp.). Each treatment had three replicates and each sample contained 20 eggs. To avoid contamination with genomic DNA, RNA samples were treated with RNase-free DNAse (Roche Diagnostics GmbH, Mannheim, Germany).

2.4.2. Fluorogenic real-time qPCR

The complete cDNA sequence of *Hsp*70 was cloned by using a RT-PCR amplification method (Genbank accession no. AY299637). The partial cDNA sequence of *Hsp*90 was obtained from EST data on the migratory locust (Kang et al., 2004). Based on the cDNA sequences of the *Hsp*70 and *Hsp*90 genes cloned above, and the 18S rRNA gene (*L. migratoria* GenBank accession no. AF370793), three primer pairs were designed (Table 1). The 18S rRNA gene was used to normalize the amounts of *Hsp*70 and *Hsp*90 mRNA. To generate cDNA from each sample, 1 μg of total RNA was performed using Superscript II (Invitrogen). *Hsp*-specific transcript standards were produced by subcloning the amplified products into plasmids (Promega). Some 10-fold serial dilutions of stock plasmids were used as quantitative standards to estimate the relative expression of each *Hsp* gene transcript; the range of standard concentrations was determined empirically. Reaction mixtures contained 1 μl of RT product, 0.5 μl of each primer (20 mM), 0.05 μl of 1500 × SYBR Green, 0.5 μl of dNTP (2.5 mM) mixture, 0.7 μl of Taq DNA polymerase (1:50) and 2 μl of 10 × buffer containing Mg^+ in 20 μl volume. For each sample, three replicates were used. Real-time PCR was performed using the iCycler iQ Real-Time Detection system intercalating SYBR Green dye from Bio-Rad Inc. according to the manufacturer's protocol. The iCycler conditions used were as follows: initial denaturation at 95 °C for 10 min, followed by 35 cycles at 95 °C for 30 s, annealing at 55 °C for 20 s, and elongation at 72 °C for 30 s. To exclude the presence of unspecific products, a melting curve analysis of products was performed routinely after amplification by a high-resolution data collection during an incremental temperature increase from 55 to 95 °C with a ramp rate of 0.2 °C/s.

Table 1
Primer pairs used for quantitative RT-PCR

Target gene	Primer pairs	Product length
*Hsp*70	ACCCAGTTATGTCGCATTTAC GGCCAATGCTTCATATCACT	159
*Hsp*90	TGGAAGAGAGGAAGATTAAAG TGGCTTAGATTCATTGTCAC	164
18sRNA	ATGCAAACAGAGTCCCGACCAGA GCGCAGAACCTACCATCGACAG	185

To verify the accuracy of the three primer pairs, PCR amplified products were sequenced.

2.5. Statistical analysis

All egg hatching data were the corrected percentage of hatched eggs. *T*-test of mean values and ANOVA were used for data analysis, and the Tukey Honest Significant Difference test procedure was used to make multiple comparisons. Probit regression and logistic regression were used to estimate the LT_{50} using SPASS software.

3. Results

3.1. Comparison between F_1 generation and parental generation

For the low temperature treatment at −5 °C, the LT_{50} of the F1 generation reared in the laboratory did not

differ from that of the parental generation collected in the field for both H and L populations (H population: $t = 1.103$, df = 4, $P = 0.332$; L population: $t = 1.531$, df = 4, $P = 0.201$) (Fig. 1). Similarly, no significant difference was found for heat tolerance (the LT_{50} at 42 °C) between the laboratory and field generations (L population: $t = 0.147$, df = 4, $P = 0.89$; H population: $t_4 = 1.558$, df = 4, $P = 0.194$) (Fig. 1). Therefore, laboratory conditions did not appear to affect the thermotolerance of the locust eggs.

3.2. Inheritance of cold tolerance

Inheritance of cold tolerance differed among the three stages of locust egg development (Fig. 2). The cold tolerance of stage I, as assayed by estimates of LT_{50} at −5 °C showed no significant variation between the two parental populations and the two reciprocal hybrids ($F_{3,11} = 3.46$; $P = 0.07$). In stage II, there were significant differences among the four stocks ($F_{3,11} = 202.24$; $P < 0.0001$): the LT_{50} of the L population was significantly greater than that of the H population ($P < 0.01$), but the two hybrids did not differ from each other ($P > 0.05$). The LT_{50} for both hybrids was approximately intermediate between their parental stocks, but with a bias towards the H population (Fig. 2). The backcross progeny exhibited similar heritable patterns (Fig. 3), indicating that nuclear

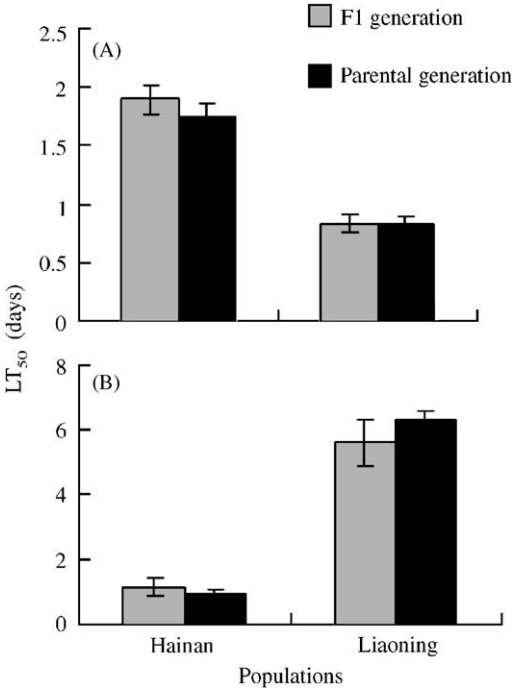

Fig. 1. Time taken to achieve 50% mortality (LT_{50}) (mean ± SE) of locust eggs of parental generation and F1 generation of both populations, Haihan and Liaoning, at stage II at 42 and −5 °C, respectively. (A) Indicates heat tolerance (42 °C) and (B) indicates cold tolerance (−5 °C).

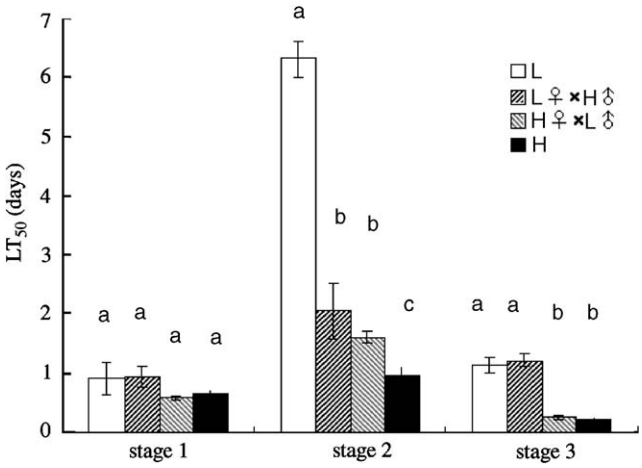

Fig. 2. LT_{50} at −5 °C (mean ± SE) of locust eggs from H (Hainan) population, L (Liaoning) population, and two reciprocal hybrids at three developmental stages. Stages I–III refer to eggs that are 2, 7, 12 days old at 30 °C. Data in the same stage group that have different letters above their corresponding bars are significantly different (Tukey's HSD. $P < 0.05$).

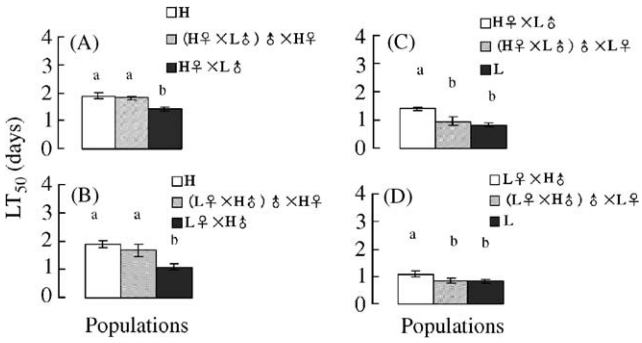

Fig. 3. LT_{50} (mean ± SE) at −5 °C of locust eggs for H (Hainan) population, L (Liaoning) population, reciprocal (L♀ × H♂; H♀ × L♂) and backcross (hybrids obtained by inbreeding between females of parental strains and males of reciprocal strains) hybrids at stage II. Stage II refer to eggs that are 7 days old at 30 °C. Data in the same group that have different letters above their corresponding bars are significantly different (Tukey's HSD. $P < 0.05$).

genetic factors contribute to differences in cold tolerance between populations rather than cytoplasmic factors, and that the genotype of the H population dominates over the L population in determining the cold tolerance of the hybrids crossed between two populations. In stage III, there were significant differences among four stocks ($F_{3,11} = 107.3$; $P < 0.01$), but the cold tolerance of each reciprocal hybrid was similar to their maternal origins, indicating a possible maternal effect.

3.3. Geographic variation and inheritance of heat tolerance

There were significant differences in heat tolerance between H and L populations in all three developmental

stages (stage I: $F_{1,5} = 13.04$; $P < 0.05$; stage II: $F_{1,5} = 136.58$; $P < 0.01$; stage III: $F_{1,5} = 10.92$; $P < 0.05$). The LT_{50} of the H population at 42 °C was greater than that of the L population in each stage (Fig. 4), indicating that, as with cold tolerance, geographical variation existed in heat tolerance between the tropical (H) and temperate (L) populations of the migratory locust.

Heritable patterns of heat tolerance in the three stages were generally consistent (Fig. 4). The LT_{50} of both reciprocal hybrids at 42 °C were similar to those of their maternal parents in each stage. There were significant differences between the two hybrids in stage II ($P < 0.01$), but not in stages I and III. Similar results were found in the backcross experiments (Fig. 5), indicating that genetic variation in heat tolerance may be due to maternal or cytoplasmic factors rather than nuclear factors.

3.4. Accumulation of Hsp70 and Hsp90

To examine the patterns of *Hsp*70 and *Hsp*90 mRNA levels under heat- and cold-shock, a fluorogenic real-time qPCR analysis was performed. Under normal developmental conditions (30 °C), no significant variation in *Hsp*70 ($t = 0.936$, df = 4, $P = 0.402$) and Hsp90 ($t = 0.806$, df = 4, $P = 0.079$) mRNA expression between the two populations was observed. There were apparent differences in the cold- and heat-shock responses between the tropical (H) and the temperate populations (L) (Fig. 6). After heat shock (40 °C × 12 h) and cold shock (0 °C × 12 h), levels of *Hsp*70 mRNA in the L population did not statistically differ compared with the control (eggs incubated at 30 °C) ($F_{2,8} = 3.456$, $P = 0.1$); however, there were significant variations in the H population ($F_{2,8} = 28.71$; $P < 0.01$). Levels of *Hsp*70 mRNA in the H population increased 2.3-fold following cold shock, but no change was observed after heat shock (Fig. 6A). Levels of *Hsp*90 mRNA in the L population increased about two-fold after heat shock, but did not significantly change in the H population. Levels of *Hsp*90 mRNA in the L population were not influenced by cold shock, but increased 3.8-fold in the H population (Fig. 6B).

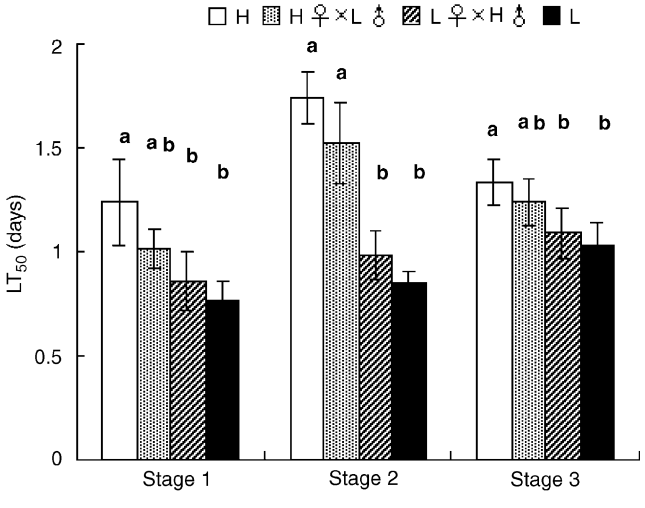

Fig. 4. LT_{50} (mean ± SE) at 42 °C of locust eggs from H (Hainan) population, L (Liaoning) population, and two reciprocal hybrids at three developmental stages. Stages I–III refer to eggs that are 2, 7, 12 days old, respectively, at 30 °C. Data in the same stage group that have different letters above their corresponding bars are significantly different (Tukey's HSD, $P < 0.05$).

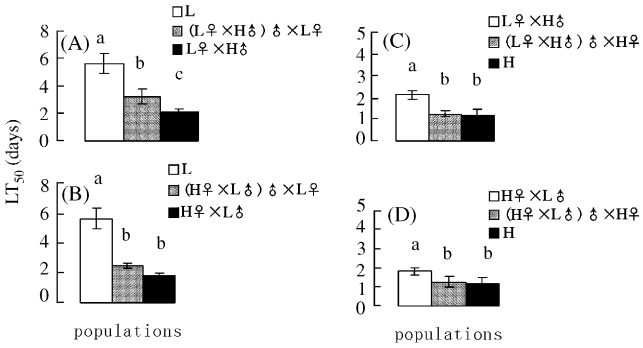

Fig. 5. LT_{50} (mean ± SE) at 42 °C of locust eggs from H (Hainan) population, L (Liaoning) population, reciprocal (L♀ × H♂; H♀ × L♂) and backcross hybrids (hybrids obtained by inbreeding between females of parental strains and males of reciprocal strains) at stage II. Stage II refers to eggs that are 7 days old at 30 °C. Data in the same group that have different letters above their corresponding bars are significantly different (Tukey's HSD, $P < 0.05$).

4. Discussion

Cold tolerance of the migratory locust varies clinically along latitudinal gradients in China (Jing and Kang, 2003). But, geographic variations detected in natural populations may have either a genetic component, or a component that is plastic in nature because thermotolerance can be influenced by prior exposure to altered temperature, photoperiods, and conditions that induce a diapause state (usually linked to photoperiod) (Huey and Berrigan, 1996). To investigate if thermotolerance of the migratory locust has a genetic component, two populations (tropical and temperate) of the migratory locust were collected in field. Although this experimental design was adequate for crossing experiments because there were great differences of cold tolerance between both populations (Jing and Kang, 2003), this approach could not test variation within local populations (Hoffmann et al., 2003; Felsenstein, 2002).

Our results indicate that phenotypic variation of thermotolerance is genetically controlled and appears to have a complex genetic basis. For cold tolerance, maternal effects or cytoplasmic factors in stage III were found. In stage II, it is nuclear genetic effects rather than

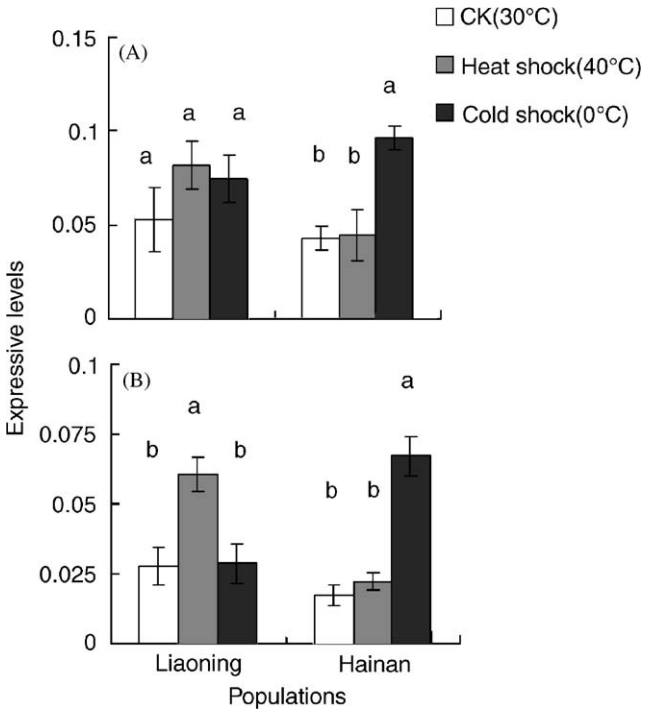

Fig. 6. Relative amounts of *Hsp*70 (A) and Hsp 90 (B) mRNA in 7 day-old eggs from L (Liaoning) population and H (Hainan) population of *L. migratoria* after they were exposed to 40 and 0 °C for 12 h, respectively. Controls were those eggs incubated for 7 days old at 30 °C. Data in the same group that have different letters above their corresponding bars are significantly different (Tukey's HSD, $P<0.05$).

a maternal effect that contributes to differences in the cold tolerance between the populations, and the genotype of the tropical population appears dominant to the genotype of the Liaoning population. There were similar between-population dominance effects in *M. sanguinipes* from reciprocal-grouped crosses made between a highly migratory population (New Mexico) and a sedentary population (Colorado) (McAnelly, 1985). For the heat tolerance, maternal effects or cytoplasmic factors appeared to be involved in all three stages. A reciprocal difference is also evident for diapause induction in *L. migratoria* (Tanaka, 1994) and for resistance to desiccation in *Drosophila* (Hercus and Hoffmann, 1999). Genetic variation of thermotolerance suggests the existence of a past evolutionary history of adaptation to local thermal environment among both Hainan and Liaoning populations. The same pattern was also observed in two intertidal fishes, *Scartichthys viridis* and *Girella laevifrons* (Pulgar et al., 2005). In addition, other properties of the locusts differ considerably between the tropical and the temperate populations. For example, the two populations studied differ in embryonic diapause, life cycle, and numerical and molecular characteristics (Kang and Chen, 1991; Tanaka, 1994), which is consistent with our results in respect to the thermotolerance.

Cold tolerance of both reciprocal crosses in egg stage II was intermediate, suggesting apparently nuclear genetic. However, for heat tolerance of locust eggs, we are unable to separate many possible ways that maternal origin can affect offspring survival because the gender of locust eggs cannot be discerned. Maternal effects have been observed for a variety of traits in many insects that are heavily influenced by maternal proteins and mRNAs, the inheritance of organelles, or micro-organisms in the cytoplasm (Mousseau and Fox, 1998). Further investigations will be needed to understand clearly the mechanisms of the maternal effect.

Different levels of tolerance to thermal stress were found among the different developmental stages of locust eggs in Hainan and Liaoning populations. Cold tolerance was observed to be greatest in stage II in both populations (Jing and Kang, 2004). The heat tolerance of stage II was greatest in Hainan populaiton, but no differences were found among the three stages in Liaoning population. Similarly, many studies have reported that thermotolerance is largely independent in different life cycle stages of *Drosophila* species (Loeschcke and Krebs, 1996; Hercus et al., 2000). For *D. melanogaster*, only 6% of preblastoderm embryos (1–2 old) hatch after exposure to 37 °C for 40 min, as compared to 85% after the onset of gastrulation (5 h and above) (Bargh and Arking, 1984). Independence of thermal adaptations between different developmental stages may reflect the fact that heat and cold tolerance are determined by different mechanisms in insects (Cossins and Bowler, 1987). For locust eggs, embryonic development can be divided into 23 stages and great changes of morphology and physiology occur during the embryonic formation from germ cells to larvae (Uvarov, 1966). In northern China, overwintering eggs remain in the soil for 6 or 7 months from the autumn until the next spring in the soil. Stage II of egg development if the overwintering stage in northern China, whereas, warmer temperatures are experienced throughout during the year in southern China (Zhang and Li, 1999). Therefore, different developmental stages of the locust eggs experience different thermal stresses in these two populations. In relative terms, heritability of heat tolerance was more consistent across the three stages, but cold tolerance varied among the different stages. The complex genetic basis of thermotolerance may reflect the fact that there were different evolutionary responses to thermal extremes across different developmental stages of the locust eggs.

Two heat shock protein genes (*Hsp*70 and *Hsp*90) of the migratory locust were cloned and used to investigate the mRNA expression of *Hsp*s with relation to thermotolerance in natural populations. We found that these two *Hsp*s were differentially expressed in migratory locust populations. After cold shock (0 °C), the levels of *Hsp*70 and *Hsp*90 mRNA increased in Hainan

population, but no variation was observed in the Liaoning population. In contrast, heat shock (40 °C) enhanced the expression of *Hsp*90 transcripts in Liaoning population, but not in Hainan population. Levels of *Hsp*70 mRNA did not vary in either population after heat shock. These results suggest that *Hsp*s expression may affect thermotolerance in natural populations of the migratory locust. In many species, sub-lethal temperatures can often induce the greatest amounts of Hsp mRNA (Feder and Hofmann, 1999). The 0 and 40 °C are sub-lethal temperatures for locust eggs (Jing and Kang, 2003) and can induce increase in *Hsps* expression (Li et al., 2000). Thus, we selected both temperatures to test for differences of *Hsps* mRNA expression between L and H populations. Lyashko et al. (1994) suggested in human fibroblasts that the heat-resistant phenotype induces larger amounts of Hsp70 than the heat-intolerant one, not at the level of *Hsp*70 mRNA induction but rather at later stages, including RNA transport to polysomes and/or other aspects in post-transcriptional regulation. Heat- and cold-shock response at the protein level should be further investigated to understand its importance in the thermotolerance of population adaptation.

A correlation has also been shown between *Hsp*s expression, stress tolerance, and gradients of environmental stress by other studies (Feder and Hofmann, 1999). For example, in *Drosophila* species selected for thermotolerance or collected from subtropical regions, there was a reduced expression of Hsp70 mRNA and protein after heat shock compared with non-selected or temperate strains (Krebs and Feder, 1997b; Goto and Kimura, 1998; Bettencourt et al., 1999; Yocum, 2001; Zatsepina et al., 2001). A relatively cold-water, northern species of mussel (*Mytilus trossulus*) also has a lower threshold for Hsp70 expression than its congener, *M. galloprovincialis*, a warm-water species (Hofmann and Somero, 1996). Although *Hsp*90 gene transcription is generally regarded to be less dependent upon thermal stress than other genes of the HSP family (Buchner, 1999), the *Hsp*90 mRNA of locust eggs was inductively expressed by heat- and cold-shock, as observed in several other Insecta (Landais et al., 2001). Studies in *Drosophila* larvae transformed with extra copies of the *Hsp*70 gene indicated that high levels of Hsps, if chronically expressed, are detrimental to the organism and would be negatively selected in nature (Krebs and Feder, 1997a; Feder and Hofmann, 1999; Zatsepina et al., 2001). Besides, morphological variation that has occurred between tropical and temperate populations (Wang and Kang, unpublished data) might be related to differentiation in constitutive expression levels of the *Hsp*90 gene between two populations, since the work on *Drosophila* and *Arabidopsis* showed links between changes in Hsp90 levels and the expression of novel morphological variation (Yahara, 1999; Queitsch et al., 2002). However, further experiments are required to understand the relationship between morphological variation and Hsp90 expression after thermal stress in the migratory locust.

In summary, a complex genetic component seems to be involved in differences of thermotolerance between Haian (tropical) and Liaoning (temperate) populations in the migratory locust. Thermotolerance of locust eggs varies across several developmental stages. Variations in regulation of *Hsps* transcription likewise may be explained by temperature-mediated selection. Further studies will be needed to investigate genetic architecture associated with thermotolerance of locust eggs and the effects of Hsps variation at the protein level.

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References

Ayrinhac, A., Debat, V., Gibert, P., Kister, A.G., Legout, H., Moreteau, B., Vergilino, R., David, J.R., 2004. Cold adaptation in geographical populations of Drosophila melanogaster: phenotypic plasticity is more important than genetic variability. Functional Ecology 18, 700–706.

Baldaia, L., Maisonhaute, C., Porcheron, P., Best-belpomme, M., 1987. Effect of heat shock on protein synthesis in *Locusta migratoria* epidermis. Archives of Insect Biochemistry and Physiology 4, 225–231.

Bargh, S., Arking, R., 1984. Developmental profile of the heat shock response in early embryos of Drosophila. Journal of Experimental Zoology 231, 379–391.

Beerli, P., Felsenstein, J., 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152, 763–773.

Beerli, P., Felsenstein, J., 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *N* subpopulations by using a coalescent approach. Proceeding of the National Academy of Sciences, USA 98, 4563–4568.

Bettencourt, B.R., Feder, M.E., Cavicchi, S., 1999. Experimental evolution of Hsp70 expression and thermotolerance in *Drosophila melanogaster*. Evolution 53, 484–492.

Brugge, J.S., Erikson, E., Erikson, R.L., 1981. The specific interaction of the Rous sarcoma virus transforming protein, pp60src, with two cellular proteins. Cell 25, 363–372.

Buchner, J., 1999. HSP90 & Co.—a holding for folding. Trends Biochemistry Science 24, 136–141.

Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. Journal of Insect Physiology 47, 649–660.

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

Feder, M.E., 1996. Ecological and evolutionary physiology of stress proteins and the stress response: the *Drosophila melanogaster*

model. In: Johnston, I.A., Bennett, A.F. (Eds.), Animals and Temperature: Phenotypic and Evolutionary Adaptation to Temperature. Cambridge University Press, Cambridge, pp. 79–102.

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

Feder, M.E., Krebs, R.A., 1997. Ecological and evolutionary physiology of heat-shock proteins and the stress response in *Drosophila melanogaster*: complementary insights from genetic engineering and natural variation. In: Bijlsma, R., Loeschcke, V. (Eds.), Environmental Stress, Adaptation and Evolution. Birkhauser, Basel, pp. 155–173.

Felsenstein, J., 2002. Contrasts for a within-species comparative method. In: Slatkin, M., Veuille, M. (Eds.), Modern Developments in Theoretical Population Genetics. Oxford University Press, Oxford, pp. 118–129.

Goto, S.G., Kimura, M.T., 1998. Heat- and cold-shock responses and temperature adaptations in subtropical and temperate species of Drosophila. Journal of Insect Physiology 44, 1233–1239.

Hercus, M.J., Hoffmann, A.A., 1999. Desiccation resistance in interspecific crosses: genetic interactions and trait correlation. Genetics 151, 1493–1502.

Hercus, M.J., Berrigan, D., Blows, M.W., Magiafoglou, A., Hoffmann, A.A., 2000. Resistance to temperature extremes between and within life cycle stages in *Drosophila serrata*, *D. brichii* and their hybrids: intraspecific and interspecific comparisons. Biological Journal of the Linnean Society 71, 403–416.

Hoffmann, A.A., Parsons, P.A., 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, New York, pp. 24–40.

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

Hofmann, G.E., Somero, G.N., 1996. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence form the heat-shock response and protein ubiquitination. Marine Biology 126, 65–75.

Huey, R.B., Berrigan, D.A., 1996. Testing evolutionary hypothesis of acclimation. In: Johnston, I.A., Bennett, A.F. (Eds.), Animals and Temperature: Phenotypic and Evolutionary Adaptation. Cambridge University Press, Cambridge, pp. 205–237.

Jing, X.H., Kang, L., 2003. Geographical variation in egg cold hardiness: a study on the adaptation strategies of the migratory locust *Locusta migratoria* L. Ecological Entomology 28, 151–158.

Jing, X.H., Kang, L., 2004. Seasonal changes in the cold tolerance of eggs of the migratory locust *Locusta migratoria* L. Environmental Entomology 33, 113–118.

Kang, L., Chen, Y.L., 1991. The analysis of numerical taxonomy to the interrelationships among different geographic populations of *Locusta migratoria* L. phase *Solitataria* (Orthoptera, Acrididae). Sinozoologia 8, 71–81.

Kang, L., Chen, X.Y., Zhou, Y., Liu, B.W., Zheng, W., Li, R.Q., Wang, J., Yu, J., 2004. The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. Proceeding of the National Academy of Sciences, USA 101, 17611–17615.

Krebs, R.A., Feder, M.E., 1997a. Deleterious consequences of Hsp70 over expression in *Drosophila melanogaster* larve. Cell Stress & Chaperones 2, 60–71.

Krebs, R.A., Feder, M.E., 1997b. Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster*, and its correlation with tolerance of ecologically relevant thermal stress. Evolution 51, 173–179.

Landais, I., Pommet, J.M., Mita, K., Nohata, J., Gimenez, S., Fournier, P., Devauchelle, G., Duonor-Cerutti, M., Ogliastro, M., 2001. Charaterization of the cDNA encoding the 90 kDa heat-shock protein in the Lepidoptera *Bombyx mori* and *Spodoptera frugiperda*. Gene 271, 223–231.

Leather, S., Walters, K., Bale, J., 1993. The Ecology of Insect Overwintering. Cambridge University Press, Cambridge, pp. 75–143.

Li, B., Cai, H., Chen, Y., 2000. HSP70KD related and temperature acclimated proteins synthesis in the migratory locust, *Locusta migratoria* (L.). In: Li, D.M. (Ed.), Chinese Entomology Towards to 21st Century. Chinese Science and Technology Press, Beijing, pp. 296–302.

Liu, J.P. (Ed.), 1995. Sudies on Acridoids of Hainan Island. Tianze Press, Xi'an, pp. 5–14.

Loeschcke, V., Krebs, R.A., 1996. Selection for heat-shock resistance in larval and in adult *Drosophila Buzzatil*: comparing direct and indirect responses. Evolution 50, 2354–2359.

Lyashko, V.N., Vikulova, V.K., Chernicov, V.G., Ivanov, V.I., Ulmasov, K.A., Zatsepina, O.G., Evgen'ev, M.B., 1994. Comparison of the heat shock response in ethnically and ecologically different human populations. Proceeding of the National Academy of Sciences, USA 91, 12492–12495.

Ma, S.J., 1962. The structure and transformation of the oriential locust breeding areas. Acta Entomologica Sinica 11, 17–30.

McAnelly, M.L., 1985. The adaptive significance and control of migratory behavior in the grasshopper *Melanoplus sanguinipes*. In: Rankin, M.A. (Ed.), Contributions in Marine Science, vol. 27: Migration: Mechanisms and Adaptive Significance. The University of Texas Marine Science Institute, Port Aransas, TX.

Mousseau, T.A., Fox, C.W. (Eds.), 1998. Maternal Effects as Adaptations. Oxford University Press, New York, pp. 54–66.

Nover, L., Scharf, K.D., 1997. Heat stress proteins and transcription factors. Cellular and Molecular Life Science 53, 80–103.

Pandey, P., Saleh, A., Nakazawa, A., Kumar, S., Srinivasula, S.M., Kumar, V., Weichselbaum, R., Nalin, C., Alnemri, E.S., Kufe, D., Kharbanda, S., 2000. Negative regulation of cytochrome C-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. The EMBO Journal 19, 4310–4322.

Pulgar, J.M., Bozinovic, F., Ojeda, F.P., 2005. Local distribution and thermal ecology of two intertidal fishes. Oecologia 142, 511–520.

Qin, W., Tyshenko, M.G., Wu, B.S., Walker, V.K., Robertson, R.M., 2003. Cloning and characterization of a member of the hsp70 gene family from *Locusta migratoria*, a highly thermotolerant insect. Cell Stress & Chaperones 8, 144–152.

Queitsch, C., Todd, A.S., Lindquist, S., 2002. Hsp90 as a capacitor of phenotypic variation. Nature 417, 618–624.

Tanaka, H., 1994. Geographic variation of embryonic diapause in the migratory locust, *Locusta migratoria* L. (Orthoptera, Acrididae), in Japan. Japanese Journal of Entomology 62, 629–639.

Travisano, M., Mongold, J.A., Bennett, A.F., Lenski, R.E., 1995. Experimental tests of the roles of adaptation, chance, and history in evolution. Science 267, 87–90.

Uvarov, S.B. (Ed.), 1966. Grasshoppers and Locusts. Cambridge University Press, Cambridge, UK.

Wang, X.H., Kang, L., 2003. Rapid cold hardening in young hoppers of the migratory locust *Locusta migratoria* L. (Orthoptera: acridiidae). Cryo-Letters 24, 331–340.

Welte, M.A., Tetrault, J.M., Dellavalle, R.P., Lindquist, S.L., 1993. A new method for manipulating transgenes: engineering heat tolerance in a complex, multicellular organism. Current Biology 3, 842–853.

Whyard, S., Wyatt, G.R., Walker, V.K., 1986. The heat shock response in *Locusta migratoria*. Journal of Comparative Physiology B 156, 813–817.

Wiech, H., Buchner, J., Zimmermann, R., Jakob, U., 1992. HSP90 chaperones protein folding in vitro. Nature 358, 169–170.

Yahara, I., 1999. The role of HSP90 in evolution. Genes to Cells 4, 375–379.

Yocum, G.D., 2001. Differential expression of two *HSP*70 transcripts in response to cold shock, thermoperiod, and adult diapause in the Colorado potato beetle. Journal of Insect Physiology 47, 1139–1145.

Zatsepina, O.G., Velikodvorskaia, V.V., Molodtsov, V.B., Garbuz, D., Lerman, D.N., Bettencourt, B.R., Feder, M.E., Evgenev, M.B., 2001. A *Drosophila melanogaster* strain from sub-equatorial Africa has exceptional thermotolerance but decreased Hsp70 expression. Journal of Experimental Biology 204, 1869–1881.

Zhang, Z.B., Li, D.M., 1999. A possible relationship between outbreaks of the oriental migratory locust (*Locusta migratroria manilenisis* Meyen) in China and the E1 Nino episodes. Ecological Research 14, 267–270.