Changes in Expression of Heat Shock Proteins in *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Relation to Developmental Stage, Exposure Time, and Temperature

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ABSTRACT The use of elevated temperatures or heat treatments for managing insect pests in food-processing facilities is becoming a popular alternative to methyl bromide fumigation. We found that young larvae (first instars) of red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), were relatively more tolerant to elevated temperatures than eggs, old larvae, pupae, and adults. Our objective was to determine whether heat shock proteins (HSPs) confer thermotolerance in *T. castaneum* life stages. Western blot analyses by using HSP 70 antibody showed constitutive expression of HSP 70 in all stages of *T. castaneum*. However, the expression of HSP 70 in young larvae increased by ≈33%, after the larvae were exposed to 40°C for 1 h. The expression of HSP 70 in other stages did not vary significantly, but for eggs the expression of HSP 70 showed significant reduction at 40°C. Young larvae possessed two distinct HSPs with molecular masses of 70 and 24 kDa, both recognized by a monoclonal anti-bovine brain HSP 70 antibody. Our study suggests that increased thermotolerance in young larvae could be due to increased expression of HSP 70 at higher temperatures. Time- and temperature-dependent expression of HSP 70 showed that the increased thermotolerance in young larvae might last as long as 8 h at 40°C or 30 min at 46°C.

KEY WORDS *Tribolium castaneum*, heat treatment, thermotolerance, heat shock proteins

THERMOTOLERANCE, OR THE ABILITY to withstand elevated temperatures in organisms, including insects, is attained by genetic adaptation, long-term thermal acclimation, and rapid heat hardening (Hallman and Denlinger 1999). One of the physiological changes an organism undergoes during the process of developing thermotolerance is the expression of heat shock proteins (HSPs) (Bendena et al. 1991, Currie and Tufts 1997, Eckwert et al. 1997, Denlinger and Yocum 1999, Lewis et al. 1999, Singh and Lakhotia 2000, Nadeau et al. 2001, Lakhotia et al. 2002, Qin et al. 2003, Sejerkilde et al. 2003).

A range of environmental stresses, including heat (Schlesinger 1990, Currie and Tufts 1997), cold (Goto and Kimura 1998), desiccation (Tammariello et al. 1999), trace metal exposure (Sanders et al. 1991, Williams et al. 1996), organic pollutants (Sanders et al. 1991), UV exposure (Nepple and Bachofen 1997), osmolarity (Kultz 1996), and anoxia (Myrmel et al. 1994) have been reported to induce HSPs in various organisms. HSPs are present and highly conserved among plants, bacteria, and insects (Eckwert et al. 1997). The enhanced synthesis of HSPs in insects immediately after subjecting cells to a stress, such as high temperature, was first reported in 1962 in the salivary glands from the fruit fly *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Grover 2002).

There are several major families of HSPs, which are classified based on their molecular mass. These include HSP 90, HSP 70, HSP 60 (chaperonin), and others with low molecular masses. For example, the HSP 70 family includes proteins that range in molecular masses from 65 to 75 kDa (Lewis et al. 1999). Members of HSP 70 are generally the most prominent proteins expressed after exposure to an environmental stress (Nadeau et al. 2001). The genes that encode HSP 70 are highly conserved in all organisms and among developmental stages within organisms (Lindquist and Craig 1988, Nadeau et al. 2001). In contrast to HSP 70, the lower molecular mass proteins are not highly conserved and are selectively expressed in certain developmental stages of an organism (Bendena et al. 1991).

Heat shock proteins play a major role in protection and maintenance of many fundamental cellular functions (Fink 1999, Nadeau et al. 2001). They play a vital role in embryonic development (Ali et al. 1996), cell differentiation, cell cycles, and hormonal stimulation (Schlesinger 1990). In addition, they act like chaperonins to maintain regular proteins in a folded state

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(Currie and Tufts 1997, Lewis et al. 1999), allow newly formed proteins to translocate across cell membranes (Sejerkilde et al. 2003), fold and assemble polypeptides (Schlesinger 1990), minimize the aggregation of non-native proteins, and target non-native or aggregated proteins for degradation and removal from cells (Feder and Hofmann 1999, Nadeau et al. 2001). Minimizing the aggregation and removal of denatured proteins are presumably the most important functions in coping with environmental stresses.

Methyl bromide, a space fumigant used widely in food-processing facilities, is scheduled to be phased out in the United States by 2005 because of its adverse effects on stratospheric ozone layer (Anonymous 1992, 1993; Makhijani and Gurney 1995). Our laboratory has been exploring the use of elevated temperatures to kill stored-product pests as an alternative to methyl bromide fumigation (Mahroof et al. 2003a, Roesli et al. 2003). Experiments in growth chambers at constant temperatures between 42 and 60°C showed that young larvae (first instars) of red flour beetle, *Tribolium castaneum* (Herbst), an important pest associated with food-processing facilities (Sinha and Watters 1985, Mills and Pedersen 1990), were relatively more heat tolerant than eggs, old larvae, pupae, and adults (Mahroof et al. 2003b). We hypothesized that HSPs might be conferring thermotolerance in *T. castaneum* life stages, and designed experiments to understand HSP induction in relation to developmental stage, exposure time, and temperature. Our specific objectives were to determine the presence of HSP 70 in eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* exposed to elevated temperatures, quantify HSP 70 levels among life stages, and characterize time- and temperature-dependent HSP expression specifically in young larvae.

Materials and Methods

Insects. Cultures of *T. castaneum* were maintained on 95% whole wheat flour and 5% (by weight) brewer's yeast at 23°C, 60% RH, and a photoperiod of 14:10 (L:D) h. Voucher specimens (no. 159) are located in the Kansas State University Museum of Entomological and Prairie Arthropod Research, Kansas State University, Manhattan, KS. Eggs laid were collected every 2 d, and the newly hatched larvae (≤12 h) were reared on bleached flour and powdered brewer's yeast in the same ratio as described above. In all tests, the age of insects from egg laying was 2 d for eggs, 6 d for young larvae, 22 d for old larvae, and 26 d for pupae. Adults used in tests were 14 d old. Individual life stages were transferred to separate square plastic boxes (4.5 by 4.5 by 1.5 cm) with perforated lids. The lids were covered with 600-μm wire mesh screens. Each box held ≈300 mg of bleached wheat flour. Because of their small size, 500 eggs or young larvae were introduced into each box. For old larvae, pupae, or adults, 50 individuals were introduced into each box.

Determination of Stage-Specific Expression of HSPs in *T. castaneum*. Boxes with each *T. castaneum* life stage were exposed to 23°C (control), 28°C and 60% RH, or 40°C and 28% RH for 1 h in environmental growth chambers (model I-36 VL, Percival Scientific, Perry, IA). In all chambers, the photoperiod was 14:10 (L:D) h. In previous tests (Mahroof et al. 2003a), we found that the relative humidity levels had no impact on the mortality of *T. castaneum* life stages exposed to elevated temperatures during heat treatment. All experimental treatments were replicated four times. The temperature treatment of 28°C was selected because this temperature represents the ambient temperature of majority of the food-processing facilities in which *T. castaneum* is found. The 40°C treatment represents temperatures experienced by insects during the initial phase of heat treatment (Mahroof et al. 2003a, Roesli et al. 2003).

Determination of Time- and Temperature-Dependent Expression of HSPs in Young Larvae. To determine time-dependent changes in expression of HSPs, young larvae were exposed to 23 (control treatment) or 40°C for 1, 2, 4, 8, 16, and 32 h. There were six replications at each temperature–time combination. The mortality of young larvae at 1–32 h, at both temperatures, also was determined by exposing 20 young larvae in separate plastic boxes with 300 mg of bleached flour. Boxes with larvae, removed at different times from growth chambers, were held at 23°C and 60% RH in 150-ml plastic containers, each containing 40 g of whole wheat flour plus 5% (by weight) brewer's yeast. Containers were placed in the control chamber until emergence of adults. Mortality of young larvae was based on those that failed to emerge into adults. Each temperature–time combination treatment for determining the mortality was replicated twice.

The temperature-dependent expression of HSPs in young larvae was studied by exposing them to 42, 46, 50, 54, and 58°C (all at 28% RH) for 30 min, after accounting for the time taken for the flour in boxes to reach the set chamber temperature. At 42°C the time taken for the flour to reach the set chamber temperature was 15 min, whereas at 58°C it was 4 min (Mahroof et al. 2003b). The temperature range of 42–58°C corresponds to the range of temperatures observed in the Kansas State University pilot flour and feed mills subjected to heat treatments (Mahroof et al. 2003a, Roesli et al. 2003). The 30-min exposure was chosen to ensure at least 60% larval survival at 54°C (Mahroof et al. 2003b). Each treatment was replicated six times. The mortality of young larvae exposed for 30 min at 42–58°C was obtained from our previously published data (Mahroof et al. 2003b).

Sample Preparation and Total Protein Assay. Insects at different stages were immediately frozen on dry ice after exposure to different temperatures and stored at −80°C until they were used. Frozen insect samples were homogenized using a Potter-Elvehjem homogenizer with a motor driven Teflon pestle in cold 0.02 M phosphate buffer (pH 7.0) containing 0.2% (vol:vol) Triton X-100. The homogenates were centrifuged at 11,500 × g for 3 min at 4°C. The supernatants were used for total protein determination and HSP analysis. Total protein assays were performed in

triplicates by using the bicinchoninic acid assay method with a V_{max} enzyme kinetic microplate reader (Molecular Devices, Menlo Park, CA) and bovine serum albumin as a protein standard according to the method described by Zhu and Gao (1998).

Western Blot Analysis of HSPs. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of *T. castaneum* samples was carried out on 4–20% Tris-glycine precast gels (pH 8.6) by using XCell II Mini-Cell gel apparatus (Novex, San Diego, CA). Samples containing same amount of total proteins were mixed with 2× SDS sample buffer (Invitrogen, Carlsbad, CA), heated for 5 min at 85°C in a water bath, and loaded onto individual wells of the gel. Electrophoresis was carried out at 105 V for ≈2 h in 1× NU-PAGE MOPS SDS running buffer (Invitrogen). Molecular mass standards ranging from 20 to 120 kDa (MagicMark Western standard, Invitrogen) and bovine brain HSP 70 (Sigma, St. Louis, MO) also were run at the same time on each gel for determining the molecular mass of the red flour beetle HSP and serving as a positive control for HSP 70, respectively.

The SDS-PAGE resolved proteins were electrotransferred onto nitrocellulose membrane (pore size 0.2 μm, Invitrogen) at 25 V for 1.5 h by using XCell II blot module (Novex) with 1× Novex Tris-glycine transfer buffer (Invitrogen) containing 20% (vol:vol) methanol. Immunodetection of HSP was performed using WesternBreeze chemiluminescent anti-mouse kit according to manufacturer's instructions (Invitrogen). Monoclonal anti-bovine brain HSP 70 antibody produced from mouse (IgG isotype, Sigma) and anti-mouse IgG antibody conjugated with alkaline phosphatase (Invitrogen) were used as the primary and secondary antibodies, respectively. The primary antibody specifically recognizes HSP 70 of insects, bovine, human, rat, rabbit, chicken, guinea pig, nematode, and plants (Sigma). The chemiluminescent reaction was identified by, exposing an X-ray film (Kodak X-Omat AR film, Eastman Kodak, New Haven, CT) to the membrane sandwich for 2 min. The film was developed and fixed using Kodak GBX developer and fixer (Sigma).

Quantification and Statistical Analysis of HSP 70 Data. Quantification of HSP 70 was performed using a gel analysis densitometer and NucleoTech Gel Expert software (NucleoTech Corporation, Westport, CT). To correct for within replicate variation of each life stage in HSP 70 amounts, densities in treated samples were normalized to the density of the representative HSP 70 band, quantified in the control treatment (23°C). The density of HSP 70 band in the control treatment was given a value of 100% and the densities of the HSP 70 band from 28 and 40°C treatments was expressed as a percentage of the control treatment (Currie and Tufts 1997). A similar normalization procedure was carried out for time-dependent or temperature-dependent HSP expression. Relative molecular masses of all proteins were estimated based on R_f values from linear calibration curves (Snedecor and Cochran 1980). Only in the young larvae of *T. castaneum*, a protein of ≈24 kDa also was detected in the Western blots. The density of the 24-kDa protein in young larvae was quantified using densitometry analyses and normalized as explained above for the HSP 70. Then, the density of the 24-kDa protein was expressed relative to that of the control treatment.

To determine significant differences in HSPs among different temperatures, quantified protein data for each stage were subjected to one-way analysis of variance (ANOVA) by using the General Linear model procedure (SAS Institute 1999). One-way ANOVA also was used to determine significant time-dependent or temperature-dependent expression patterns of HSPs in young larvae. Treatment means were separated using the Tukey's test. The relationship between amounts of heat shock protein expressed and the time for peak expression was described using a simple linear regression. Data transformations were not deemed necessary, because data were normally distributed. All differences were considered significant at the $\alpha = 0.05$ level.

Results

Stage-Specific Expression Pattern of HSPs. In the Western blot analysis, the monoclonal anti-HSP 70 antibody recognized a conserved epitope of the HSP 70 in the eggs, young larvae, old larvae, pupae, and adults of *T. castaneum,* exposed to both control (23°C) and heat-treated conditions (28 and 40°C). The mean ± 1 SE ($n = 3$) molecular masses of HSP 70 based on R_f values for all stages was 75.1 ± 0.6 kDa for eggs, 68.8 ± 1.5 kDa for young larvae, 68.6 ± 1.9 kDa for old larvae, 68.4 ± 1.3 kDa for pupae, and 73.0 ± 1.7 kDa for adults. The molecular mass for the bovine brain HSP 70, used as a positive control, was 75 kDa based on our Western blot analysis.

The patterns of protein accumulation in control and heat-shocked eggs showed a decreasing trend with an increase in temperature (Fig. 1A). There was a decrease of ≈35% ($F_{2, 9} = 4.26$; $P = 0.04$), in the amount of HSP 70 expressed in eggs exposed for 1 h at 40°C (Fig. 2A). In young larvae, a strong HSP 70 signal was detected (Fig. 1B). The amount of HSP 70 in young larvae increased by ≈29% as the temperature increased from 23 to 28°C, and 33% as the temperature increased from 23 to 40°C (Fig. 2B). The increased amount of HSP 70 at 28 and 40°C was significantly different from expression at 23°C ($F_{2, 9} = 6.72$; $P = 0.01$). In the current study, an unexpected reactivity of the anti-bovine HSP 70 monoclonal antibody resulted in a protein band of ≈24 kDa in young larvae only (Fig. 1B). Although the identity of 24-kDa protein is undetermined, we quantified this protein in our current study (Fig. 2C). Despite the 69% increase in 24-kDa protein when the temperature was increased from 23 to 40°C, the quantified Western blot data showed no significant difference between control and heat-shocked conditions ($F_{2, 9} = 2.59$; $P = 0.10$).

The HSP 70 accumulation in old larvae, pupae, and adults (Fig. 1C–E) did not show marked differences among temperatures. Densitometry analysis of the Western blots showed no significant differences be-

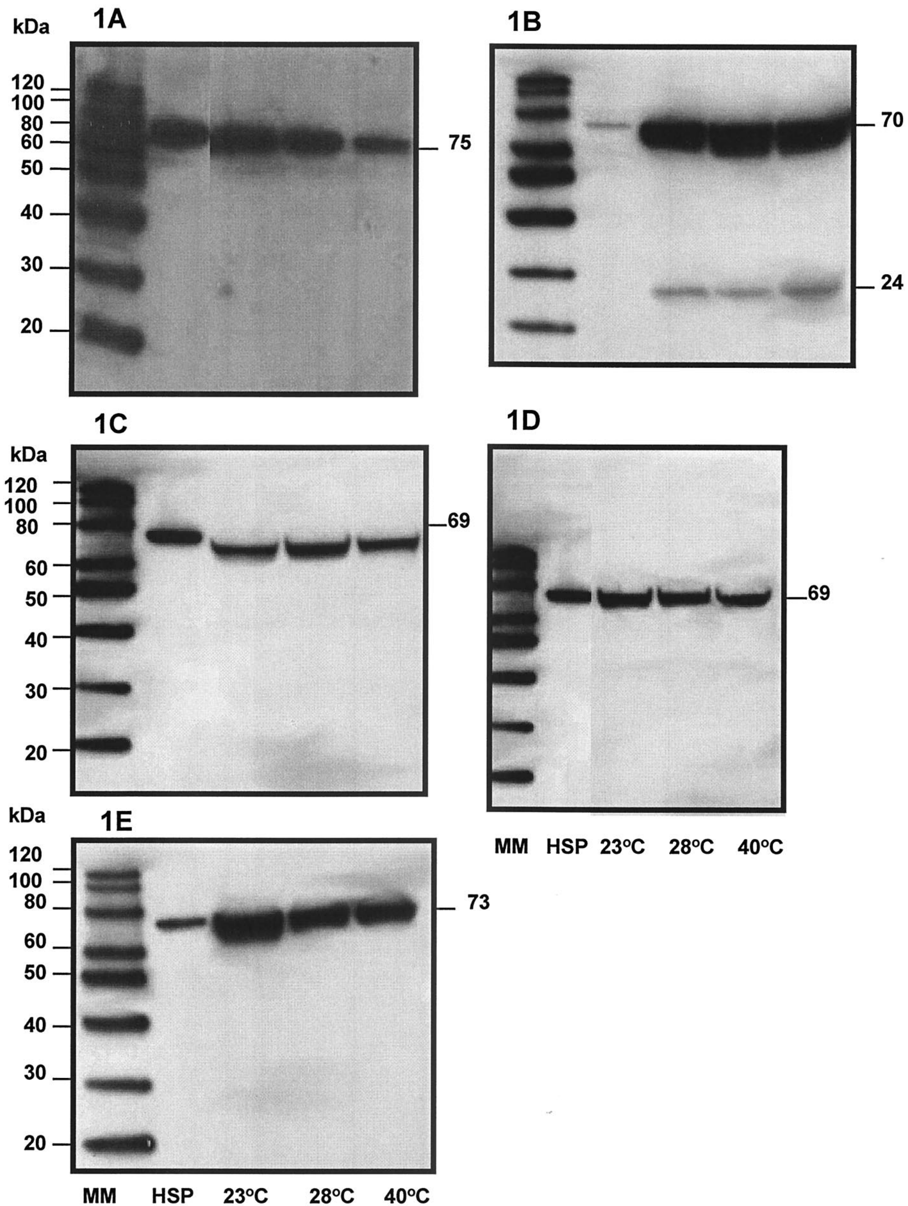

Fig. 1. Western blot analysis of HSP 70 in *T. castaneum* life stages exposed to three temperatures for 1 h. MM, molecular mass standard; HSP, HSP 70-positive sample. Lanes contain equal amounts of protein (80 μg) from homogenates of eggs (A), young larvae (B), old larvae (C), pupae (D), and adults (E).

tween the relative amounts of HSP 70 in control and heat-shocked conditions for old larvae ($F_{2,\ 9}$ = 0.62; P = 0.55; Fig. 2D), pupae ($F_{2,\ 9}$ = 2.57; P = 0.13; Fig. 2E), and adults ($F_{2,\ 9}$ = 0.76; P = 0.49; Fig. 2F).

Time-Dependent Variation in Accumulation of HSPs in Young Larvae. Mortality of *T. castaneum* young larvae exposed for 1–32 h at 40°C was minimal. The mean ± 1 SE (n = 2) mortalities at 40°C for 1-, 2-, 4-, 8-, 16-, and 32-h exposures were 0, 0, 2.5 ± 0.8, 2.5 ± 0.8, 5.0 ± 0, and 7.5 ± 0.8%, respectively. Therefore, larval survival was not adversely affected at the various exposure times at 40°C. The HSP 70 levels in young larvae exposed up to 32 h at 40°C increased steadily between 1 and 8 h, peaked at 8 h, and tended to decline after 16 h. A 117% increase in the amount of HSP 70 was observed as the time of exposure at 40°C increased from 1 to 8 h. The amount of HSP 70 observed at 8 h was significantly higher than that observed at 1, 2, 4, or 32 h at 40°C and in the control treatment at 23°C ($F_{6,\ 24}$ = 4.4; P = 0.004). The amount of HSP 70 expressed was ≈31% higher than the control treatment after 32-h exposure at 40°C (Fig. 3A). No significant time-dependent changes were observed for the accumulation of 24-kDa protein ($F_{6,\ 24}$ = 2.1; P =

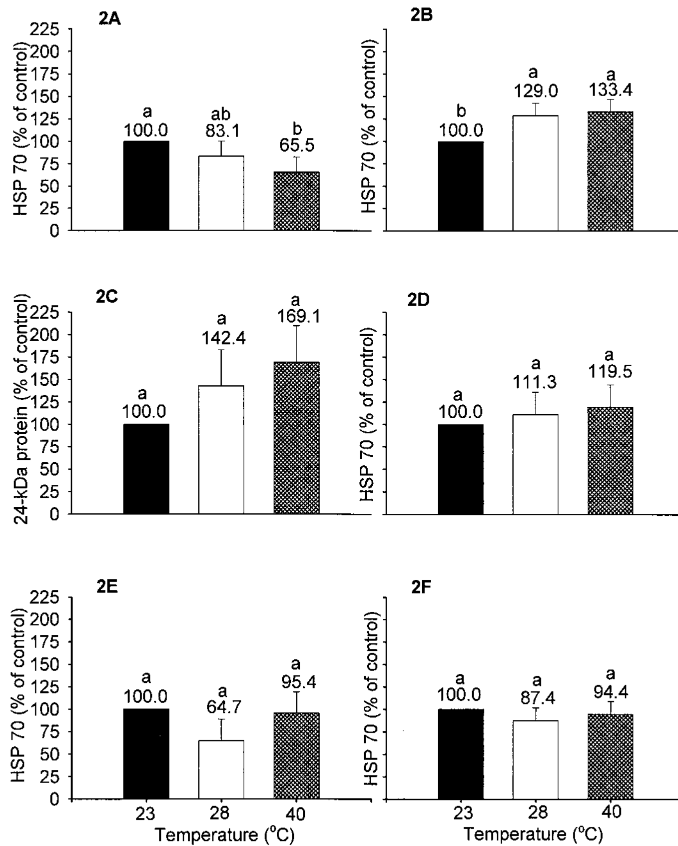

Fig. 2. Stage-specific variation in accumulation of HSP 70, expressed as a percentage of control treatment (23°C) in *T. castaneum* eggs (A), young larvae (B), old larvae (D), pupae (E), and adults (F). C represents density of 24-kDa protein measured in young larvae. Each bar represents the mean ± 1 SE for $n = 4$ replicates. Means followed by different letters are significantly different ($P < 0.05$; Tukey's test).

0.08; Fig. 3B). Regression analysis of the amount of HSPs against time revealed a positive linear relationship in HSP 70 expression during the first 8 h. Based on the slope value of the regression line, the amount of HSP 70 increased by 17.8% for every hour of exposure ($r = 0.99$, $P = 0.01$). In contrast to HSP 70, the expression of the 24-kDa protein during the first 8 h was not significant ($r = 0.74$, $P = 0.2$).

Temperature-Dependent Variation in Accumulation of HSPs in Young Larvae. The mean ± 1 SE ($n = 3$) percentage of young larvae exposed to 42, 46, 50, 54, and 58°C for 30 min was 1.0 ± 0.1, 1.5 ± 0.2, 2.0 ± 0.2, 40.0 ± 1.6 and 94.0 ± 2.3%, respectively. The level of HSP 70 increased with an increase in temperature, and was highest at 46°C and declined after 46°C, with the lowest amount observed at 58°C (Fig. 4A). The relative density of HSP 70 accumulated at 46°C was significantly different ($F_{6,\ 20} = 9.7$; $P < 0.0001$) than the density at other temperatures. Expression of the 24-kDa protein was highest at 42°C and then declined with increasing temperatures, and at 58°C the protein mostly disappeared (Fig. 4B). The relative density of the 24-kDa protein at 42°C was significantly different ($F_{6,\ 20} = 3.4$; $P = 0.02$) than that at 23, 50, 54, or 58°C.

Discussion

The temperature of 40°C for heat shock protein induction was similar to temperature used for other insects, such as the tiger swallowtail, *Papilio glaucus* (L.); tobacco hornworm, *Manduca sexta* (L.) (Fittinghoff and Riddiford 1988); flesh fly, *Sarcophaga crassipalpis* Macquart (Joplin and Denlinger 1990); grasshopper *Spathosternum prasiniferum* (Walker); American cockroach, *Periplaneta americana* (L.); and earworm *Heliothis armigera* (Hübner) (Singh and Lakhotia 2000). In the current study, eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* responded to the heat shock treatment. The molecular masses for all life stages of *T. castaneum* correspond to the HSP 70 family, because the molecular masses of HSP 70 are generally between 65 and 75 kDa (Lewis et al. 1999). The highly conserved nature of HSP 70 was confirmed because the anti-bovine brain HSP 70 monoclonal antibody detected HSP 70 in all life stages of heat-shocked *T. castaneum.* The levels of HSP 70 protein detected at 23°C (control temperature) suggest high constitutive levels of HSP 70 exist in all life stages of *T. castaneum.* Joplin and Denlinger (1990)

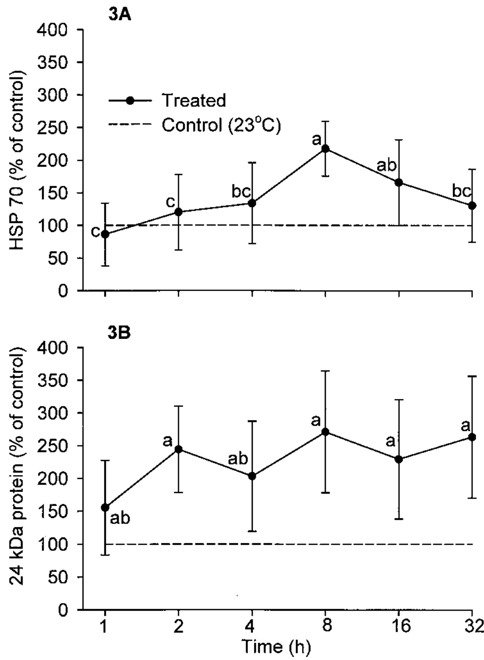

Fig. 3. Time-dependent accumulation of HSP 70 (A) and 24-kDa protein (B) in young larvae of *T. castaneum*. The horizontal broken line resembles the relative percent density of HSPs in the control treatment (23°C). Each point represents the mean ± 1 SE for $n = 6$ replicates. Means followed by different letters are significantly different ($P < 0.05$; Tukey's test).

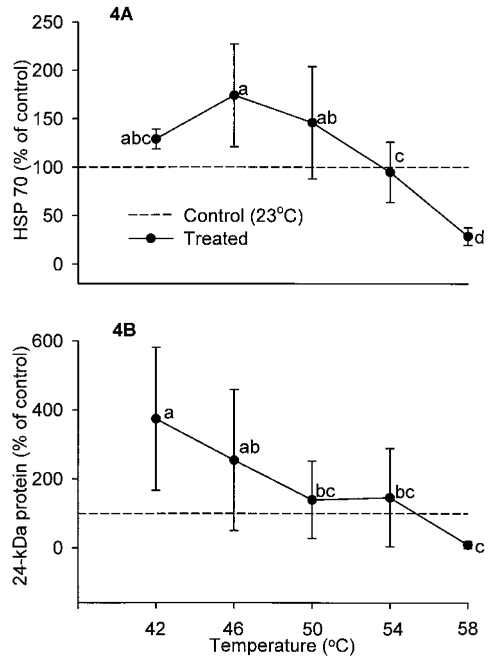

Fig. 4. Temperature-dependent expression of HSP 70- (A) and 24-kDa protein (B) in young larvae of *T. castaneum*. The horizontal broken line resembles the relative percent density of HSPs in the control treatment (23°C). Each point represents the mean ± 1 SE for $n = 6$ replicates. Means followed by different letters are significantly different ($P < 0.05$; Tukey's test).

reported that the heat shock cognate (HSC) is expressed in large quantities under nonstressful conditions. Eukaryotic cells contain a multigene family that encodes several related 70-kDa proteins (Matranga et al. 2000), which differ in their intracellular location and regulation. These 70-kDa proteins include the constitutive HSC 73 and the stress inducible HSP 72.

The levels of expression of HSP 70 varied among life stages of *T. castaneum*, which suggests independent regulation in the various life stages. The plausible reason for a decrease in HSP 70 in eggs at higher temperatures may be related to degradation, reduced synthesis, or lack of synthesis. Lang et al. (2000) observed that in the African clawed frog, *Xenopus laevis* (Daudin) *hsp 70* genes are not heat-shock inducible until after the mid-blastula transition. In contrast, constitutive *hsc 70* mRNA is detectable in the early stages, such as in the cleavage stage of embryos, indicating a maternal origin for these transcripts. A lack of heat inducibility for *hsp 70* gene expressions also has been reported in eggs of a midge, *Chironomus tentans* F. (Renier et al. 2003); *D. melanogaster* (Hass et al. 1990); an amphibian, the Spanish ribbed newt, *Pleurodeles waltl* (Michahelles); and the Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) (Zafarullah et al. 1992). The eggs used in the current study were 24–48 h old. The postblastula stage in *T. castaneum* egg occurs at or later than 60 h (Sokoloff 1974). Therefore, heat-inducible HSP 70 may not be activated in the eggs we used. Based on our study, *T. castaneum* eggs have high levels of constitutively expressed heat shock protein, possibly of a maternal origin. However, eggs exposed to heat stress do not synthesize heat-inducible HSP 70. Hence, the relatively low level of thermotolerance observed in eggs (Mahroof et al. 2003b) may be due to lack of heat-inducible HSP 70 synthesis at higher temperatures. However, Welte et al. (1993) showed that levels of thermotolerance attained in developing embryo of *Drosophila* depended critically upon the conditions on pretreatment, the developmental stage of the embryo, and severity of the heat shock.

Mahroof et al. (2003b) demonstrated that young larvae were the most heat-tolerant stage of *T. castaneum*. HSP 70 expression observed in young larvae was ≈33% higher compared with other stages. Increased levels of thermotolerance in young larvae may be due to the increased induction of HSP 70 at high temperatures. Welte et al. (1993) showed that manipulating the expression of single *hsp 70* gene itself was responsible for increased thermotolerance in a *Drosophila* strain, whereas Krebs et al. (1998) showed that developmental stages varied in the concentration of HSP 70 after heat shock. On average, first instars of the *D. melanogaster* produced ≈30% more HSP 70 than did male and female adults and wandering phase third instars. They also observed that the first instars are more heat tolerant than other stages and concluded

that inducible HSP 70 contribute to the increased thermotolerance of *D. melanogaster* first instars, but reduced the fitness by affecting larval development to adulthood.

The extra 24-kDa protein in young larvae observed in our study may have resulted from the characteristic and relatively stable breakdown products of HSP 70. For example, Lindquist (1986) reported that HSP 70 is proteolytically reduced to either ≈40–44-kDa or ≈ 18–24-kDa proteins. The breakdown products have sometimes been reported as novel HSPs in organisms. However, the reason why this protein was consistently seen only from young larvae and not from the other life stages warrants further study.

Our studies showed that the highest thermotolerance generated for *T. castaneum* young larvae at 40°C lasted for 8 h. A possible reason for the 14% reduction in the amount of HSP 70 expressed in 1 h at 40°C compared with the control treatment is not clear. Based on the results obtained in time-dependent variations in HSP 70 accumulation, it is likely that HSPs do not confer long-term thermotolerance at supraoptimal temperatures. Landry et al. (1982) showed that exposing "Morris hepatoma 7777 cell line" to 43°C for 30 min induced HSPs up to 8 h, at which thermotolerance was maximized. However, as the induced HSPs degraded over the next 60–80 h, there was a corresponding decrease in thermotolerance. In a similar study, Yocum and Denlinger (1992) reported that pretreating *D. melanogaster* for 2 h at 40°C induced 72-kDa HSP associated with a concomitant increase in thermotolerance. However, the 72-kDa HSP decreased at 48 h and disappeared after 72 h.

Temperature-dependent variation in accumulation of HSPs in young larvae shows very little HSP 70 synthesis at 58°C. The reduced synthesis is likely due to the severity of the high temperatures used. In the current study, we used a 30-min heat shock at 58°C, whereas in many others studies, the maximum exposure was 2 h at 45°C (Chen et al. 1991, Yocum and Denlinger 1992). Qin et al. (2003) reported optimum induction of HSP 70 in the migratory locust *Locusta migratoria* (L.), at temperatures between 43 and 45°C. In many species at very high temperatures, transcription and translation of *hsp 70* genes shut down. Overall, our study showed that the HSP 70 expression is stage- and time-specific, and temperature-dependent.

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