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SHORT COMMUNICATION

# Extreme thermotolerance and behavioral induction of 70-kDa heat shock proteins and their encoding genes in honey bees

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**Abstract** Foraging honey bees frequently leave the hive to gather pollen and nectar for the colony. This period of their lives is marked by periodic extremes of body temperature, metabolic expenditure, and flight muscle activity. Following ecologically relevant episodes of hyperthermia between 33°C and 50°C, heat shock protein 70 (Hsp70) expression and *hsp70*/*hsc70-4* activity in brains of nonflying laboratory-held bees increased by only two to three times baseline at temperatures 46–50°C. Induction was undetectable in thoracic–flight muscles. Yet, thorax *hsp70* mRNA (but not *hsc70-4* mRNA) levels were up to ten times higher in flight-capable hive bees and foraging bees compared to 1-day-old, flight-incapable bees, while brain *hsp70*/*hsc70-4* mRNA levels were low and varied little among behavioral groups. These data suggest honey bee tissues, especially flight muscles, are extremely thermotolerant. Furthermore, Hsp70 expression in the thoraces of flight-capable bees is probably flight-induced by oxidative and mechanical damage to flight muscle proteins rather than temperature.

**Keywords** Brain · Honey bee · Hsp70 · Muscle · Thermotolerance

## Introduction

Despite a vast literature describing the structure, function, and evolution of heat shock proteins (Hsps), very little is known about the patterns of Hsp expression throughout the lifespan of an organism in nature as well as the specific life history and environmental correlates of Hsp expression (Feder and Hofmann 1999). Best described are patterns of Hsp expression in plants and marine intertidal organisms, which are not highly mobile and can become extremely hot. Temperature increases usually occur on diurnal and/or seasonal cycles and these organisms increase Hsp expression in response (Alamillo et al. 1995; Buckley and Hofmann 2004; Burke et al. 1985; Colombo et al. 1995; Hamilton et al. 1996; Helmuth and Hofmann 2001; Hendershot et al. 1992; Hernandez and Vierling 1993; Hofmann and Somero 1996; Kimpel and Key 1985; Nguyen et al. 1994). Even fewer data exist for terrestrial animals, and those that do focus primarily on insects. Subadult *Drosophila melanogaster* encounter temperatures exceeding 40°C if the necrotic fruit they infest is sunlit and express Hsp70 in response (Feder et al. 1997; Feder 1997). However, adult *Drosophila* rarely express Hsp70 even on very hot days, presumably due to the ability to escape to thermally neutral microfugia (Feder et al. 2000). The desert ants of the genus *Cataglyphis* experience body temperatures above 50°C while foraging, prior to which expression of Hsp70 family members increases, perhaps reflecting an anticipatory response (Gehring and Wehner 1995). Naturally occurring hypothermic events (such as diapause, overwintering in exposed sites, and recovery from freezing) also coincide with Hsp expression (Denlinger 2002).

As heterothermic insects, honey bees provide a unique system within which to address these questions. Honey bees live in large colonies usually containing over 20,000 individuals, comprised of one queen and her offspring, a few hundred male drones, and thousands of female workers. Embryos and larvae are individually housed in open cells on honeycombs and cared for by adult bees that feed the larvae and maintain the environment at a remarkably

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constant 33–35°C and 70–75% relative humidity (Winston 1987). Honey bees exhibit a form of behavioral development termed "temporal polyethism," moving through a series of behaviorally defined life history stages in an age-related fashion. For the first 2–3 weeks of life, adult workers perform tasks inside the hive such as brood care and hive maintenance. Typically, at about 3 weeks of age, workers transition to performing tasks outside the hive such as foraging. As bees age and move from tasks in the hive to foraging, their behavior, physiology, sensory systems, neuroanatomy, circadian rhythms, flight performance, and predation risk all change (Elekonich and Roberts 2005; Roberts and Elekonich 2005).

Foraging honey bees are subject to several spatial and temporal gradients of heterothermia. They require a minimum thoracic–flight muscle temperature of 27°C to fly which they generate by contracting their flight muscles, but prior to departing the hive they warm up to much higher thoracic temperatures (~37°C), which permit the generation of maximum flight forces. Honey bees are moderately good thermoregulators during flight and are able to maintain the temperature of the flight muscles across a wide range of air temperatures (Roberts and Harrison 1999). Even so, tissue temperatures of flying–foraging honey bees are strongly affected by air temperature, which in turn is subject to strong diurnal and seasonal variation. During flight, thoracic temperature and head temperature can vary with air temperature by 10°C and 14°C, respectively. Each body segment can exceed 40°C, especially during flight in warm air temperatures. Furthermore, during flight at nearly all air temperatures, there is strong heterothermia *within* an individual bee, with thoracic temperature normally much higher than head temperature (Roberts and Harrison 1999). Thus, honey bees may have an intrinsic need to mitigate heat-induced damage.

Despite this implied need, only three studies have measured Hsp expression and/or the activity of their encoding genes in honey bees. Larval Hsp70 concentration increases in response to foulbrood (*Paenibacillus*) infection (Gregorc and Bowen 1999), while in vitro heat shock of the larval fat body and laboratory heat shock of adults induces the expression Hsp70s, Hsp82, Hsp90, and small Hsps (Chacon-Almeida et al. 2000; Severson et al. 1990). In a recent microarray study focusing on genetic correlates of behavioral development, Whitfield et al. (2003) reported very modest increases in mRNA levels for a variety of *hsp*s in the brains of a subset of foragers compared to hive bees. Given their endothermy and flight requirements, the modest increase of hsps in flying bees is puzzling. Thus, this study measured variation in *hsp70*/*hsc70* mRNA and Hsp70 family protein levels in the brains and thoraces of honey bees in different behavioral states and along an ecologically relevant thermal gradient in the laboratory.

## Results and discussion

### Effects of heat shock

Small Plexiglas cages containing groups of 25–30 bees obtained at eclosion from a standard field colony were exposed to temperatures between 33°C and 50°C when the bees were 9 days of age to look for effects of heat shock on mortality and tissue-specific expression of hsp70 mRNA and proteins. Decreased survivorship occurred at temperatures above 46°C (Fig. 1). At 48°C, survival was 100% between 0 and 1.5 h but dropped to 0% at 2 h. Surprisingly, 100% of nonflying caged bees survived 30 min of exposure to 50°C. After 1 h at 50°C, 65% still survived, but survivorship dropped to 0% at 1.5 h. At temperatures between 33°C (normal hive temperature) and 46°C, there was no mortality in any of the cages of bees with exposures between 0.5 to 3.0 h. Overall, these experiments suggest that honey bees are extremely thermotolerant.

Following heat exposure, the concentration of Hsp70 family proteins was significantly lower overall in thoraces than in heads ($F_{1,\ 394}=11.34$, $p<0.001$, Fig. 2). Particularly surprising was that exposure to temperatures up to 50°C

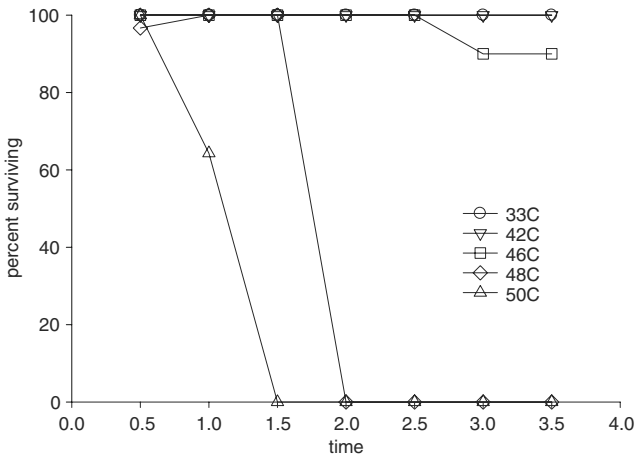


**Fig. 1** Survivorship of nonflying bees following heat exposure. Groups of 25–30 newly emerged adult worker bees from a standard field colony maintained at the University of Nevada Las Vegas were placed into small (10×10×7 cm) Plexiglas cages (as in Grozinger et al. 1999). Cages were held in an incubator (70% RH, 33°C). Bees had ad libitum access to 50% sugar syrup (*w*/*v*) that was replenished each day. When replenishing the sugar syrup, we observed bees walking, standing, grooming, and performing trophallaxis but never flying. When the bees were 9–10 days posteclosion, the cages were exposed to 38°C, 42°C, 44°C, or 50°C in a humidified incubator for periods between 0.5 and 4 h. Controls remained in the rearing incubator at 33°C (hive temperature control). The number of dead bees was recorded for each cage and the remaining live bees were collected directly into liquid nitrogen. Each point represents data from one cage of bees. There was little or no mortality in bees held at 33°C and 42°C, while the time prior to 50% mortality at 46°C, 48°C, and 50°C was 3.25, 1.75, and 1.25 h, respectively

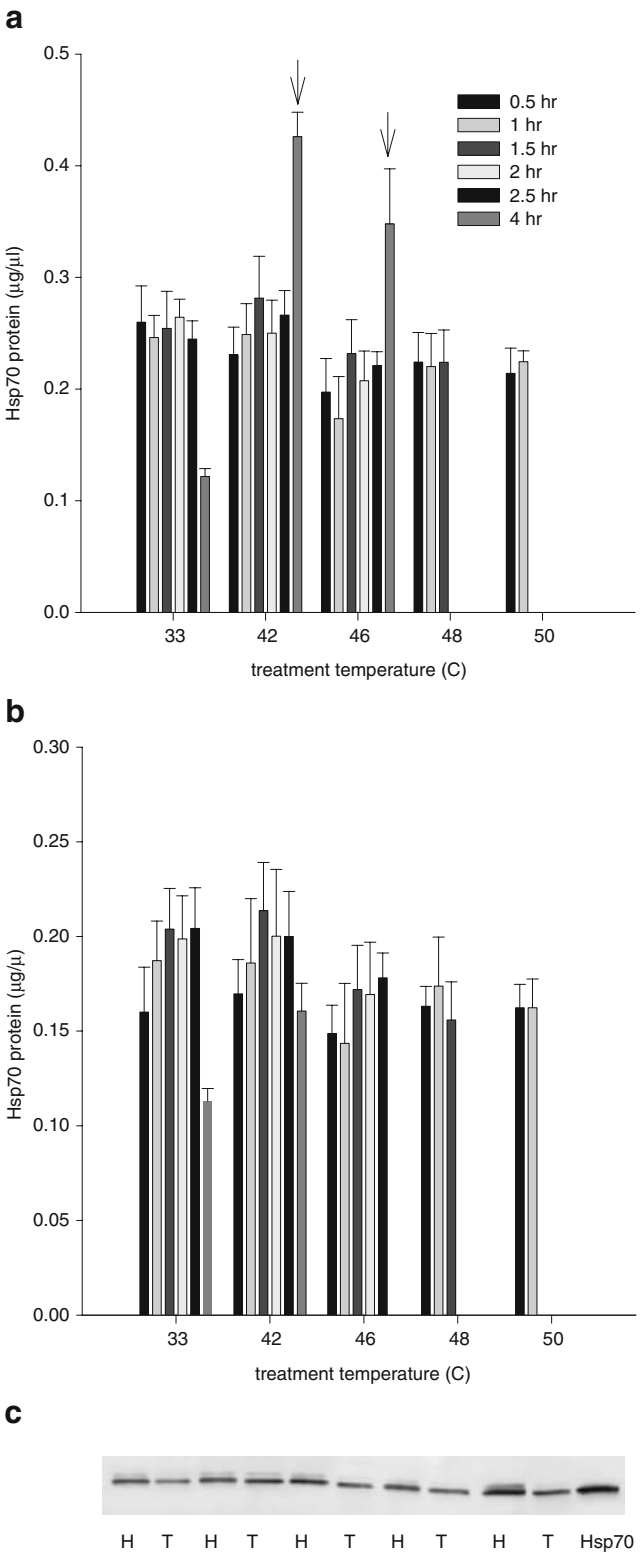


**Fig. 2** Hsp70 family proteins in 9-day-old honey bees following heat exposure in the laboratory. **a**=head; **b**=thorax. Data are means±SE from five to eight bees at each temperature. *Arrow* indicates significant differences (see text for details). **c** The representative gel shows Hsp70 family proteins in the head and thorax of one bee from each thermal treatment at 1 h of exposure. The *far right lane* of gel represents an Hsp70 protein standard (0.25 µg; from Bovine brain, Sigma H9776, Sigma-Aldrich, St. Louis, MO, USA). Frozen heads and thoraces from individual heat-exposed bees (caged heat exposure described in the Fig. 1 caption) were homogenized in lysis buffer (Tris, NaCl, Triton X-100, glycerol, and protease inhibitors; Grozinger et al. 1999). Total protein concentration was measured from an aliquot of each sample with a Bradford assay (Bio-Rad, Hercules, CA, USA). All samples were diluted to 25 µg/15 µl with Laemmli buffer (Bio-Rad, Hercules, CA, USA) to standardize the amount of protein loaded into the gel. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Bio-Rad Protean II System, Tris–glycine buffer and 10% gels) followed by transfer to a polyvinylidene fluoride filter. Blots were immunoprobed with a mouse monoclonal antibody to Hsp70 (Sigma H5147, Sigma-Aldrich, St. Louis, MO, USA) that recognizes induced and constitutively expressed Hsp70 proteins and has been used previously with honey bee tissue (Chacon-Almeida et al. 2000; Gregorc and Bowen 1999; Williams et al 2008). Following incubation with a horse-radish-peroxidase-conjugated secondary antibody, blots were visualized with ECL Plus on a Typhoon 9200 Phosphoimager (Amersham/GE Healthcrare, Piscataway, NJ, USA). Linear dilutions indicated that positive immunoreactivity was linearly related to control and sample protein concentration. Additionally, preincubation of primary antibody with the control protein completely eliminated immunoreactivity with honey bee protein

caused no increase in levels of thoracic *hsp70/hsc70-4* mRNA ($F_{4,\ 20}$=0.403, $p$>0.05, $F_{4,\ 20}$=1.994, $p$>0.05, respectively, Fig. 3) and Hsp70 family proteins (Tukey's honestly significant difference (HSD), all $p$ values>0.05, Fig. 2). In contrast, Hsp70 protein concentration in the heads of worker bees varied significantly with temperature ($F_{4,\ 199}$=4.37, $p$<0.05) and there was an interaction between exposure time and temperature ($F_{19,\ 199}$=1.74, $p$<0.05, Fig. 2). There was a slight increase after 4 h of exposure to 42°C or 46°C in brain levels of Hsp70 proteins (Tukey's HSD, $p$<0.05, Fig. 2) but not *hsp70/hsc70-4* mRNA ($F_{4,\ 20}$=0.962, $p$>0.05, $F_{4,\ 20}$=1.646, $p$>0.05 respectively, Fig. 3). Even so, this level of inducible Hsp70 expression is extremely modest compared to the roughly 1,000-fold inducibility of Hsp70 in *D. melanogaster*, an isothermic species, following a heat shock at 36°C (Velazquez et al. 1983).

## Normal expression in bees of different behavioral groups

Hsp 70 mRNA expression was also compared in brains dissected from the head tissue and thoraces of free-living worker bees from four age-matched behavioral groups from two colonies: day-old adults, nurse bees, precocious foragers, and experienced foragers. In the brain, mRNA expression for both genes differed significantly (although modestly) between behavioral groups ($F_{3,\ 45}$=5.52, $p$<0.01, $F_{3,\ 45}$=11.31, $p$<0.01 respectively, Fig. 4) and between individuals from different colonies (i.e., different genetic backgrounds; $F_{1,\ 45}$=18.433, $p$<0.001, $F_{1,\ 45}$=8.88, $p$<

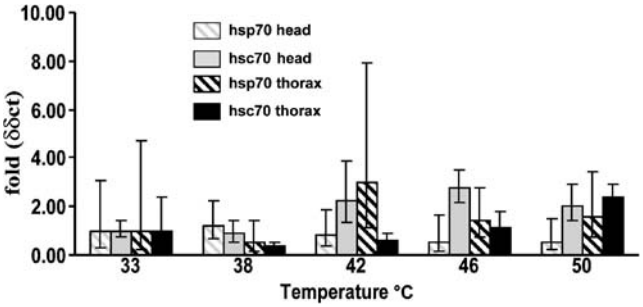


**Fig. 3** Expression of *hsp70* and *hsc70-4* mRNA following 1 h of heat exposure. *Striped bars*=*hsp70*; *solid bars*=*hsc70*. *Gray bars*=heads; *black bars*=thoraces. There were no significant differences in mRNA expression (see text for details)., $n$=5 per bar. *Hsp70* (inducible form) and *hsc70-4* (one constitutive form) mRNA were measured using quantitative real-time polymerase chain reaction (PCR) in individual brains and individual thoraces from bees collected following heat exposure as described in the Fig. 1 caption. For graphical presentation of RNA levels, we followed the $2^{-\Delta\Delta Ct}$ transformation normalized to values for bees kept at 33°C for the heat shock experiment (ABI User Bulletin 2; ABI, Foster City, CA, USA; see also Ben Shahar et al. 2002, 2003) with SE from the nontransformed values. Statistical analysis was done on nontransformed delta Ct values. Collected bees were held frozen at −80°C until tissue dissection on dry ice. RNA was extracted with the mini-RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions including on-column DNase treatment. Probes and primers were designed using Primer Express (Applied Biosystems Inc., Foster City, CA, USA) software. For measurement of RNA coding for the inducible form of honey bee Hsp70, we used primers and probes made from a 501-bp sequence whose best BLASTX match is the *Anopheles albimanus* Hsp71 protein (SwissProt P41825) with an *e* value of 4e-18 (Jay Evans, personal communication). Probes and primers for Hsc70-4 were designed from a 282aa contig sequence identified as a BLASTX match to *D. melanogaster* Hsc70-4 (Bronk et al. 2001) from the Honey Bee EST database. Levels of *hsp70* and *hsc70-4* mRNA were measured relative to the housekeeping gene ribosomal protein 49 (*rp49*), which has been used previously as a transcriptional control in honey bee and *Drosophila* tissue (Ben Shahar et al. 2002, 2003; Daborn et al. 2002; Thellin et al. 1999). *Rp49* levels did not differ significantly with temperature in heads ($F_{4,\ 20}$=0.669, $p$>0.05) or thoraces ($F_{4,\ 20}$=1.434, $p$>0.05), indicating that its transcription and/or transcripts are very thermostable in honey bees. Reverse transcription of previously extracted RNA was performed according to the manufacturer's protocols, the Omniscript Reverse Transcription Reagents Kit (Qiagen, Valencia, CA, USA; 200 ng total RNA). We monitored expression of *hsp70* and *hsc70-4* mRNA in brains and thoraces with SyberGreen. Heads and thoraces from heat-shocked bees were blocked for each plate (96 wells, Bio-Rad Icycler) so that all groups and all genes were run in any given day's assay

0.01) for *hsp70* and *hsc70-4*, respectively). However, there was no interaction between the main effect of colony and behavioral group ($F_{3,\ 45}$=0.078, $p$>0.05, $F_{3,\ 45}$= 0.156, $p$>0.05 for *hsp70* and *hsc70-4*, respectively) suggesting genotype did not influence the behavioral differences.

Although statistically apparent, differences between bees from the four behavioral groups were modest. Newly emerged 1-day-old bees, experienced foragers, and precocious foragers had significantly higher levels of brain *hsc70-4* mRNA than in the brains of hive bees ($p$<0.05, Tukey's HSD for each comparison). Levels of *hsp70* mRNA were significantly higher in experienced foragers and 1-day-old bees than in the brains of hive bees ($p$<0.05, Tukey's HSD for each comparison). In comparison, levels of *hsp70*mRNA did not differ significantly between precocious foragers and age-matched hive bees ($p$>0.10 Tukey's HSD).

Brain mRNA measures suggest that the modest increase in Hsp70 protein under heat stress may be due to *hsc70* transcription rather than *hsp70* transcription. This is consistent with previous research showing the constitutive or induced expression of specific family members may vary with species, tissue, and developmental stage (Joplin and Denlinger 1990; Palter et al. 1986; Singh and Lakhotia 2000). In contrast, expression did not change dramatically in the brains of bees in different behavioral states. Although levels of both mRNAs were significantly lower in the brains of hive bees compared to newly eclosed 1-day-old bees or foragers, this change is very modest with high individual variation. Therefore, it may have little functional consequence.

In contrast to expression in brains, levels of thoracic *hsc70-4* mRNA did not differ significantly among behavioral groups ($F_{3,\ 33}$=1.225, $p$>0.5). But thoracic *hsp70* mRNA concentration varied significantly among behavioral groups ($F_{3,\ 33}$=3.03, $p$<0.05, Fig. 4). Thoracic *hsp70* mRNA was most abundant in the thoraces of experienced foragers and was significantly higher (up to tenfold) than in 1-day-old bees who cannot fly (Tukey's HSD, $p$<0.05). However, thoracic *hsp70* mRNA expression in those experienced foragers did not differ significantly from expression in the thorax of precocious foragers or flight-capable hive bees (Tukey's HSD, $p$>0.05). Yet previous work indicates that foragers express roughly twice as much Hsp70 family proteins in their thoraces as hive bees (Roberts and Elekonich 2005). *Hsc70-4* expression in the thorax did not differ significantly between the colonies ($F_{1,\ 33}$=0.182, $p$>0.05) but *hsp70* expression was significantly different in the two colonies ($F_{1,\ 33}$=4.68, $p$<0.05). There was no interaction between the behavioral group and colony effects for either gene ($F_{3,\ 33}$=1.086, $p$>0.05, $F_{3,\ 33}$=0.317, $p$>0.05 for *hsp70* and *hsc70-4*, respectively) suggesting that colony differences were not responsible for the behavioral group effects in the thorax as well.

One explanation for these results may be that thoraces, but not heads, become extremely hot during foraging trips and thus thoraces, more specifically flight muscles, have evolved exceptional thermostability relative to head tissues, showing less Hsp70 protein and mRNA expression in response to heat shock and higher levels of hsp70 mRNA with flight. In *Drosophila*, an isothermic insect, the brains of *Drosophila* are fairly thermotolerant while the gut is less

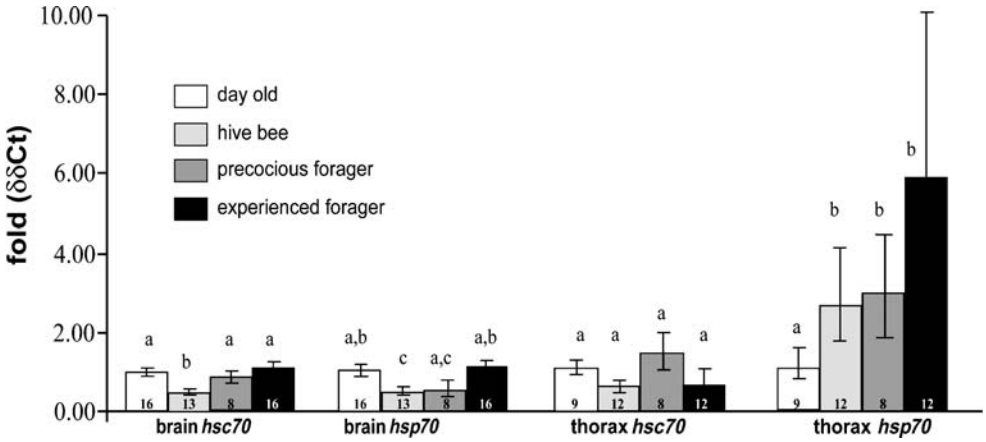


**Fig. 4** Levels of *hsp70* and *hsc70-4* mRNA in the heads and thoraces of honey bees from different behavioral groups from typical field colonies. Sample sizes are in the *bars*. Different *letters* indicate significant difference between behavioral groups within each gene × tissue group. Graphical presentation of RNA levels follows the $2^{-\Delta\Delta Ct}$ transformation presented in ABI User Bulletin 2 (ABI, Foster City, CA, USA; see also Ben Shahar et al. 2002, 2003) normalized to values for 1-day-old bees with SE from the nontransformed data. We analyzed the untransformed quantitative real-time PCR data with analysis of variance using group and colony as main effects also followed by post hoc Tukey's HSD tests (Wilkinson et al. 1996). *Hsp70* (inducible form) and *hsc70-4* (one constitutive form) mRNA was measured using quantitative real-time PCR in individual brains and individual thoraces from age-matched bees representing four age–behavioral groups: day-old adults, nurse bees, precocious foragers, and experienced foragers. Collections of age–behavioral groups were made at the University of Illinois Urbana-Champaign from July 23–September 13, 2001. Daytime air temperatures during the collection period ranged from 12°C to 33°C; however, hive temperatures remained relatively constant between 33°C and 35°C. *Collection details*: 1 week prior to the introduction of known age bees, active foragers (indicated by the presence of pollen or distended abdomens reflecting nectar foraging) were paint-marked on the abdomen (Testors PLA) upon returning to the hive. Three days later, these "experienced foragers" were collected at the hive entrance upon their return from a subsequent foraging trip. These bees all had signs of wing wear indicating that they had been actively foraging for some time. Bees typically begin foraging at an average age of 23 days posteclosion (range 7–40, Winston 1987) so these bees were likely >3 weeks old. The remaining groups, "day-old" bees, "age-matched hive bees," and "precocious foragers" originated from two frames of bees in capped cells which were about to emerge from metamorphosis obtained from each of three hives and placed in an incubator (33°C, 70% RH) overnight. Of the bees that emerged from the comb the next day, 25 individuals were immediately collected into liquid nitrogen as samples of 1-day-old newly emerged bees. The remaining bees were paint-marked to reflect their emergence date and hive of origin and subsequently returned to their hives. Older foragers were removed to induce a subset of these marked bees to begin foraging early, as "precocious foragers." At 12–14 days of age, the marked bees were collected at the entrance of each of the three hives as they returned from a foraging trip. At the same time, marked bees performing brood care were collected off the honey comb as "age-matched hive bees," sisters to the "precocious foragers." Brains from bees in the behavioral groups were dissected from the head capsule on dry ice under ×40 magnification (Olympus SZH10 stereo microscope) homogenized into 500 μl of Trizol buffer (Invitrogen) following Schulz et al. (2003) and stored at −80°C until RNA extraction using the mini-RNeasy kit with on-column DNase treatment (Qiagen, Valencia, CA, USA). RNA was reverse-transcribed using the ABI Reverse Transcription Reagents Kit (100 ng of total RNA) according to the manufacturer's protocols. For qualitative real-time PCR, all brain samples were run in duplicate loaded on the same reaction plate (384 wells, one plate per gene, ABI Prism7900HT sequence detector). Thoraces from the same individuals were run in triplicate and blocked by groups for each plate (96 wells, Bio-Rad Icycler). Primers and probes for *hsp70*, *hsc70-4*, and *rp49* were tagged with either SyberGreen or Tamra/FAM (Icycler or ABI Prism, respectively). Although the brains and thoraces all came from the same individual bees collected at UIUC and held frozen until analysis, the brains were analyzed at UIUC and the thoraces were analyzed at UNLV after the author's relocation. Due to the equipment available, mRNA expression in brains and thoraces were measured with slightly different methods at UIUC and UNLV as described above. Thus, expression levels were only compared within each tissue (i.e., brains and thoraces were not compared to each other). There were no significant differences among behavioral groups in brain or thoracic *rp49* levels ($F_{3,\ 45}=1.35$, $p>0.05$; $F_{3,\ 33}=2.63$, $p>0.05$, respectively), confirming its effectiveness as a transcriptional control (Ben Shahar et al. 2002)

so with a lower threshold Hsp70 induction temperature (Krebs and Feder 1997). Honey bees may represent an example of spatial tissue heterothermia *within an organism* acting as a selective agent upon the regulation of Hsps and their encoding genes.

Bees are one of the few endothermic insects and use contraction of the flight muscles to produce body heat (Heinrich 1980, Winston 1987). Honey bees regulate actively flight muscles between 35°C and 47°C during foraging, and head temperatures rarely exceed 42°C, even during flight in the hottest summer days. Abdomens are cooler yet, differing little from ambient temperatures (Roberts and Harrison 1999). Although thoraces also contain digestive tract tissue, respiratory trachea, and some neural tissue, 90% of the thorax by volume consists of flight muscle suggesting that the expression profiles primarily reflect expression in muscle. However, there is no thoracic Hsp70 induction when nonflying bees are held in the laboratory at similar temperatures (Fig. 1) suggesting that levels observed in free-flying experienced foragers are due to flight and endothermically produced muscle temperatures rather than ambient temperature. Thus, tissue differ-

ences in the thermal kinetics of *hsp70* and *hsc70-4* mRNA and Hsp70 family proteins in nonflying bees likely reflect differences in the normal operating temperature between tissues and reflect chaperone activity due to extreme protein degradation, repair, maturation, and replacement needed by the heavily taxed forager flight muscles (conservatively estimated to contract over four million times per day; Winston 1987; Harrison et al. 1996). If so, then other indicators of stress such as carbonyls indicating protein damage, antioxidant activity, ubiquitinization, and proteolysis should be elevated in flight muscle, particularly during or just after foraging. Ongoing experiments suggest that antioxidant expression and protein damage may indeed increase over the course of foraging efforts throughout the day (Williams et al. 2008).

Subsequently, the high variance in forager *hsp70* levels likely reflects the variation in individual foragers' time per trip and/or number of trips taken that day. Once a worker begins foraging, an age-dependent decay in glycogen synthesis occurs (perhaps due to overuse damage) limiting total flight performance to about 800 km (Neukirch 1982). Because the experienced foragers were the oldest bees we collected, the increased variance in *hsp70* mRNA levels may reflect the effects of differential aging due to differences in flight history. Within the hive, the bees maintain the temperature between 33°C and 35°C with body heat produced by flight muscle contractions (Winston 1987) so hive bees, precocious foragers, and experienced foragers would all experience similar temperatures when in the hive itself regardless of external ambient temperatures. Collections of precocious and experienced foragers overlapped making it unlikely that the differences in variance are due to atmospheric temperature encountered outside the hive. Furthermore, summer temperatures in Illinois were relatively moderate and cool compared to the temperatures of laboratory-heated bees which caused only a relatively modest induction of *hsp70* mRNA.

While it is possible that bees from hives in Illinois and those in Las Vegas might have population-related differences in Hsp70 mRNA and protein levels, it is unlikely since these are not wild bees. Both University of Illinois at Urbana-Champaign (UIUC) and University of Nevada, Las Vegas (UNLV) hives are derived from standard European honey bees, *Apis mellifera*. In North America, managed honey bees are derived from a mix of European subspecies, most typically *A. mellifera ligustica*. Importation of bees was banned in the early 1900s until recently; thus, most American stocks are related. Furthermore, queen bees for both sets of colonies were purchased from many of the same commercial sources. In managed hives, queen bees are typically replaced each year and beekeepers manage the hives to prevent colony reproduction by swarming. Thus, there is seldom an F2 generation. So it is unlikely that the hives either in Illinois or Las Vegas had developed any heritable local adaptations.

Although thoraces appear to be specialized for high-temperature operation and thus show little induction of *hsp70* mRNA during exposure to heat stress, we cannot rule out that the effect of heat stress on either tissue might be mitigated by another heat shock protein as different Hsp family members can be differentially induced in different tissues. For example, exposure of flesh flies, *Sarcophaga crassipalpis*, to heat stress induces Hsp65 in the flight muscle while Hsp72 increases in the brain and integument (Joplin and Denlinger 1990). Honey bees are known to have members of the Hsp70, Hsp90, Hsp40, Hsp60, and Hsp20 families (Honey Bee Genome Sequencing Consortium 2006; Chen et al 2006, Elekonich, unpublished) any of which could be involved in the response to heat stress.

Given their evolution in tropical climates, endothermy, and colony thermoregulation at 33–35°C (Winston 1987), the general insensitivity as demonstrated by the low mortality rates of honey bees upon exposure to high temperature is not surprising. Other thermophilic species have a higher threshold temperature for Hsp70 expression than more temperate congeners (Hofmann and Somero 1996; Tomanek and Somero 1997; Huey and Bennett 1990; Gehring and Wehner 1995). A major remaining question is how diurnal and seasonal variation in thermal stress and flight activity–history affects Hsp activity in this endothermic, behaviorally complex species. Seasonal changes in Hsp activity are well documented in gobiid fishes (Buckley and Hofmann 2004) and both seasonal and circadian changes in expression and induction occur in plants in both north temperate and desert ecosystems (Burke et al. 1985; Colombo et al. 1995; Hamilton et al. 1996). Experiments are underway to identify the role of muscle temperature versus muscle activity on Hsp expression and protein damage in foraging bees and to separate the roles of behavioral state, behavioral activity, age, and environmental context including social, seasonal, and circadian influences on multiple markers of cellular stress.

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