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Research Article

Study of gene expression of Heat shock proteins HSP-70 and some physiological and immunological aspects of three types of heat-tolerant and non-tolerant insects

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Abstract
Heat shock proteins (HSPs), particularly HSP-70, are critical to insect adaptation under extreme environmental stress. This study evaluated HSP-70 gene expression in three insect species-*Apis mellifera* (honeybee), *Camponotus xerxes* (ant), and *Musca domestica* (housefly)-subjected to heat stress (45°C, 50°C, 55°C) and extreme cold (liquid nitrogen, −96°C). Gene expression was measured using RT-PCR, and fold changes were calculated relative to the control groups. In *A. mellifera*, HSP-70 expression increased from a baseline of 1.54-fold in controls to 3.00-, 3.30-, and 6.09-fold at 45°C, 50°C, and 55°C, respectively; exposure to liquid nitrogen induced a 4.41-fold increase. In *C. xerxes*, expression rose from 1.03-fold (control) to 3.07-fold, 3.67, and 7.69-fold with increasing temperatures, while nitrogen exposure led to a 5.61-fold rise. *M. domestica* exhibited the highest expression at 55°C (5.62-fold) and after nitrogen exposure (4.94-fold), compared to 1.01-fold in controls, although expression at 45°C and 50°C was lower (2.04- and 1.91-fold, respectively).The results indicated that all three species upregulate HSP-70 in response to thermal and cold stress, with the greatest expression observed at 55°C and upon exposure to liquid nitrogen. Notably, *C. xerxes* showed the strongest heat-induced response, while *M. domestica* exhibited pronounced expression under both heat and cold, suggesting robust thermal adaptation. These findings underscore the species-specific dynamics of HSP-70 regulation and its pivotal role in cellular protection and survival under extreme environmental conditions.

Keywords: Ant, Heat stress, Honeybee, Housefly, HSP70 gene expression, Thermal adaptation

INTRODUCTION

Heat shock proteins (HSPs) are a conserved group of proteins that play a crucial role in cellular responses to environmental stress, particularly heat stress. These proteins function as molecular chaperones, aiding in the proper folding of proteins, preventing protein aggregation, and facilitating protein repair following stress-induced damage. Among them, HSP-70 is one of the most extensively studied and significant members due to its crucial role in protecting cells and maintaining cellular function under various stress conditions (Bai *et al*., 2021). HSP-70 is known for its ability to bind to unfolded or damaged proteins, thereby preventing their aggregation and facilitating their proper refolding. Additionally, it plays a role in protein transport across cellular membranes, the degradation of damaged proteins, and the regulation of cellular signalling pathways. The importance of HSP-70 extends to various cellular processes, including stress response, cell cycle regulation, and apoptosis, making it an essential component in maintaining cellular stability and function (Wang *et al*., 2021). Insects are ectothermic organisms, meaning their body temperature is directly influenced by their surrounding environment. This makes them susceptible to temperature fluctuations, whether increases or decreases, which can impact their physiological functions, development, and survival. In this context, HSP-70

plays a pivotal role in helping insects adapt to thermal changes by providing cellular and protein protection against heat stress-induced damage (Jin *et al*., 2020).

Upon heat exposure, insects rapidly upregulate the HSP-70 gene, increasing intracellular protein levels to manage damaged proteins by preventing aggregation and aiding in repair or degradation. HSP-70 also stabilizes cell membranes and preserves protein integrity, maintaining vital functions under stress. Beyond heat, it responds to oxidative stress, heavy metals, and infections, underscoring its key role in insect cellular defense. (Zhang *et al*., 2015).

Genetic studies on HSP70, conducted through hybridisation with the *Drosophila* HSP70 gene, revealed that this gene contains a 1.9-kb open reading frame encoding a 70-kDa protein. The promoter region features interleaved heat shock elements (HSEs) (Tan, 2023). Other studies on the mollusk Nucella ostrina have found that heat stress induces the highest levels of HSP70, which in turn affects metabolic activity and growth rates (Gill *et al*., 2023). HSP70 also functions as a molecular chaperone, facilitating the transport of proteins within cells. It has been investigated as a novel adjuvant in vaccines targeting tumor cells and enhancing resistance to viral infections (Cifric *et al*., 2024). HSP70 provides significant protection against tissue damage caused by hypoxia, as it is highly expressed in cardiac endothelial cells, which are more sensitive to heat stress than other cardiac cells (muscular or neural) (Brunt and Minson, 2021). Furthermore, Jurcau *et al*. (2024) reported that HSP70 is primarily located in neurons and glial cells in the brain rather than in vascular structures. The protein's protective role may involve different cell types and intercellular interactions across organs. In cardiovascular tissues, HSP70 has critical cardioprotective applications, as safeguarding blood vessels from free radical damage extends protection to entire organs (Singh *et al*., 2024).

Honeybees are crucial pollinators responsible for fertilizing approximately 80% of flowering plants and 75% of trees while producing honey. Ants, renowned for their resilience, contribute to soil quality improvement through the turnover of organic matter and the dispersion of seeds, while also acting as predators that control insect populations. Houseflies, despite being vectors of pathogens, play a crucial role in the decomposition of organic matter and the pollination of plants. The present study aimed to investigate the expression patterns of HSP-70 in three insect species under varying thermal conditions.

MATERIALS AND METHODS

Collection of insects used

In this study, three insect species-honeybees (*Apis mellifera*), ants (*Camponotus xerxes*), and houseflies (*Musca domestica*) were selected due to their ecological relevance and varied environmental adaptations. They were collected from natural environments to ensure they had adapted to ambient temperatures. Random sampling was conducted for all species, collecting 125 specimens per species. Honeybees were collected from an apiary in Anbar Province, Iraq, using insect nets, and then placed in ventilated glass containers. Ants were collected from various locations, including desert areas near the University of Anbar, using tweezers and manually placed in glass containers.

Houseflies were sampled from butcher shops in Ramadi city, Anbar Province, using insect nets and stored in glass containers. Specimens were then transferred to the laboratory for further study and identification at the Natural History Museum, University of Baghdad, Iraq.

Study design

Insects were divided into five replicates, with 25 specimens of each species placed in ventilated glass containers and exposed to controlled temperature treatments for 30 minutes as follows:

Control group (untreated)

Liquid nitrogen-exposed group (flash-frozen and homogenized)

Heat-exposed groups: incubated at 45°C, 50°C, and 55°C, then homogenized. Trizol reagent was added post-homogenization for molecular analysis.

Molecular study

Primers used in the Study

Primers were designed using Primer3Plus (version 4), a web-based tool for primer design, and verified through comparisons with the University of California Santa Cruz (UCSC) Genome Browser and the National Centre for Biotechnology Information (NCBI) database. The primers were synthesized and lyophilised by Alpha DNA Ltd., Canada. Table 1 provides the sequences used in this study.

Total RNA extraction

Total RNA was extracted from all samples using the TransZol Up Plus RNA Kit (TransGen Biotech, ER501-01; TransGen Biotech Co., Ltd., Beijing, China), following the manufacturer’s instructions.

Complementary DNA (cDNA) Synthesis

Total RNA was converted into complementary DNA (cDNA) using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech Co., Ltd., Beijing, China), following the manufacturer’s instructions.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Gene expression levels were assessed using the quantitative real-time polymerase chain reaction (qRT-PCR) technique, a highly sensitive method for determining the levels of stable RNA. The SYBR Green assay was

Table 1. Primer sequences used in the study

Primer	Sequence (5'→3' direction)	Primer size bp	Product size bp	Ta °C
Hsp70 (Gene Expression)				
Forward	CACCAAGCAGACCCAGAC	18	116	58
Reverse	CGAACTTTCCGAGGAGGT	18		
Housekeeping gene Actin				
Forward	ATGGTCGGCATGGGACAG	18	153	58
Reverse	GAGTTCATTGTAGAAGGTGT	21		

employed to confirm gene expression. The target gene primers were designed and sequenced by Alpha DNA Ltd., Canada, lyophilized, and stored at -20°C. The mRNA levels of the internal control gene (*HSP70*) and the reference gene *ACTIN* were amplified (Schmittgen & Livak, 2008).

Quantitative Real-Time PCR (qRT-PCR) Runs

Gene expression levels and fold changes of *HSP70* were evaluated using the TransStart® Top Green qPCR SuperMix kit. The cycle threshold (Ct) value was measured to determine gene expression levels for each sample. The required volume for each reaction component was determined based on the data in Table 2.

Thermal Profile

The reaction was programmed using the optimal thermal profile, as shown in the table below:

RESULTS AND DISCUSSION

The present study demonstrated clear species-specific differences in HSP70 gene expression in response to thermal and cold stress, highlighting distinct adaptive strategies among *Apis mellifera, Camponotus xerxes*, and *Musca domestica*. While all three insect species upregulated HSP70 expression upon exposure to elevated temperatures and liquid nitrogen, the magnitude and pattern of expression varied significantly.

Table 2. Components of the Real-Time PCR reaction for *HSP70* and *ACTIN* gene expression

Component	Volume (µl)
2× TransStart® Top Green qPCR Super Mix	10
Nuclease-free water	6
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
cDNA	2

Table 3. Thermal profile for the expression of *HSP70* and *ACTIN* genes

Step	Temperature (°C)	Time (sec)	Cycles
Enzyme activation	94	30	1
Denaturation	94	5	40
Annealing	58	34	
Extension	72	20	

Apis mellifera exhibited a moderate and progressive increase in HSP70 expression, peaking at 6.09-fold at 55°C. This response reflects the honeybee's well-developed yet temperature-sensitive defense mechanism. Being a eusocial insect with thermoregulatory behaviors within the hive, the honeybee's reliance on HSP70 under extreme conditions suggests a molecular backup system when behavioral thermoregulation is insufficient (Zhao *et al*., 2022). In contrast, *Camponotus xerxes* exhibited the highest HSP70 expression overall, particularly at 55°C (7.69-fold), indicating a robust cellular defence mechanism that is well-suited for survival in arid and fluctuating desert environments. This aligns with recent findings on thermophilic ant species, where elevated HSP70 levels are strongly linked to ecological dominance in heat-stressed habitats (Nguyen *et al*., 2021). Interestingly, *Musca domestica* demonstrated a biphasic response, with moderate induction at 45°C and 50°C, followed by a sharp increase (5.62-fold) at 55°C. It also showed relatively high expression (4.94-fold) under liquid nitrogen, suggesting broad-spectrum resilience. This plasticity is consistent with recent studies showing that synanthropic insects, such as houseflies, rapidly modulate HSP expression in response to urban stressors, including chemical and thermal fluctuations (Zhu *et al*., 2023).

Compared to prior work, this study offers novel insights by simultaneously evaluating gene expression in three functionally and ecologically distinct insect species under both heat and cryogenic stress. Most earlier studies have focused either on a single species or limited environmental variables (e.g., only heat stress), often overlooking the importance of cold stress or comparative evaluation (Ali *et al*., 2021).

The regulation of *HSP70* gene expression primarily depends on the activation of the heat shock factor (*HSF1*), which, upon exposure to thermal or chemical stress, translocates from the cytoplasm to the nucleus. There, it binds to regulatory elements in *HSP* genes, leading to an increase in the production of this protective protein at the molecular level. The regulatory mechanisms of HSP70 expression include post-translational modifications, such as phosphorylation and histone activation, which facilitate chromatin relaxation and enhance the transcription process. Gu Ling-Ling *et al*. (2019) reported that HSP70 gene expression

is not merely a short-term response, but can undergo regulatory modifications that influence cellular stress responses over extended periods, supporting the role of HSP70 in long-term cellular adaptation mechanisms.

The present results showed a significant increase in *HSP70* gene expression in honeybees exposed to stress conditions. In the control group, where no environmental stress was applied, gene expression remained relatively low due to the absence of external pressures. However, expression levels significantly increased following exposure to liquid nitrogen cooling and extreme temperatures, particularly at 55°C. These findings align with those of Zhou *et al*. (2020), who observed increased HSP70 gene expression in the white-backed planthopper (Sogatella furcifera) subjected to abiotic stressors, such as extreme temperatures and heavy metals. *HSP70* plays a crucial role in protecting and repairing proteins, ensuring cell survival and functionality under thermal and chemical stress conditions.

The relatively low *HSP70* expression levels in the control group suggest a basal level of activity for the protein under normal conditions, reflecting cellular stability in the absence of environmental stress. However, exposure to liquid nitrogen cooling stimulated the expression of defensive genes, such as HSP70, leading to a statistically significant increase. When exposed to thermal stress at 45–50°C, *HSP70* expression was also induced, albeit to a lesser extent than in the liquid nitrogen-treated group, indicating the activation of cellular protective mechanisms to prevent protein damage and maintain cellular stability under shifting environmental stressors from chemical to thermal stress (Table 4).

The highest *HSP70* gene expression level was observed at 55°C, indicating a strong response to extreme heat stress. At this temperature, protective pathways are activated at a high level to prevent cellular collapse and ensure survival. This increase is crucial for mitigating potential protein damage resulting from the accumulation of reactive oxygen species (ROS) at high temperatures. The results confirm that both thermal and chemical stress significantly induce *HSP70* gene expression as part of the cellular response to maintain function. Expression levels increase progressively with greater stress intensity, demonstrating the honeybee's adaptability to environmental challenges.

Additionally, the study observed a higher gene expression under extreme conditions, indicating that both high cooling and severe heating elicit stronger responses compared to moderate heat exposure. The elevated response under these extreme conditions suggests that honeybees possessed robust adaptive mechanisms to cope with intense thermal fluctuations. These findings support the conclusion that *HSP70* is one of the most critical defensive proteins, produced in large quantities during heat stress to stabilize proteins and prevent cellular and metabolic damage.

Social insects, such as honeybees, exhibit a strong heat stress response through the production of *HSP70*, which enhances their resilience to harsh environmental conditions. Additionally, chemical stress, such as sudden cooling, triggers an acute heat shock protein response, particularly in the group treated with liquid nitrogen. Furthermore, Meng *et al*. (2022) demonstrated that *Arma chinensis*, a predatory stinkbug, shows significantly elevated HSP70 expression under both high and low temperature stress . This parallels our findings in honeybees, supporting the idea that extreme thermal fluctuations—including rapid cooling and intense heating—elicit stronger HSP70 responses than moderate heat exposure.

Relative estimation of *HSP70* gene expression in *Camponotus xerxes* ants

Camponotus xerxes is a social insect that exhibits an exceptional ability to withstand harsh environments. This unique ant species is highly adaptable to extreme conditions, playing an essential ecological role as it thrives in desert and semi-desert regions. It is commonly found in hot and arid environments, demonstrating remarkable adaptation to limited-resource habitats. This species serves as a valuable model in studies aimed at understanding physiological and molecular mechanisms of adaptation to thermal stress.

HSP70 protein reflects cellular responses to environmental changes, where its gene expression is induced by thermal or chemical stress. This protein plays a criti-

Table 4. Showing *HSP70* gene expression levels in honeybees under different temperature treatments

Group	Mean Ct (Housekeeping Gene)	Mean Ct (HSP70)	ΔCt (Mean Ct HSP70 − Ct Housekeeping)	Fold of gene expression
Control (No exposure to stress)	22.21	36.88	14.66	1.54
Nitrogen (−96°C, liquid nitrogen)	19.31	32.20	12.89	4.41
45°C (Moderate heat stress)	23.59	36.87	13.28	3.00
50°C (High heat stress)	18.91	31.90	12.99	3.30
55°C (Extreme heat stress)	20.25	32.42	12.17	6.09

Ct -Cycle threshold

Table 5. Showing the gene expression levels of *HSP70* in *Camponotus xerxes* under different temperature conditions

	Camponotus xerxes			
Groups	**Means Ct value of Housekeeping Gene**	**Means Ct value of HSP70**	**ΔCt (Means Ct value of HSP70)**	**Fold of gene expression**
Control (No exposure to stress)	18.36	35.77	17.41	1.03
Nitrogen Exposed to liquid nitrogen, −96°C)	18.64	33.64	15.00	5.61
45°C (Moderate heat stress)	18.46	34.43	15.96	3.07
50°C (High heat stress)	18.76	34.31	15.55	3.67
55°C (Extreme heat stress)	18.17	32.70	14.52	7.69

Ct -Cycle threshold

cal role in protecting cells from damage caused by the accumulation of misfolded proteins, thereby enhancing the insect's survival in extreme environmental conditions.

The results in Table 5 illustrate the gene expression levels of *HSP70* in *Camponotus xerxes* ants exposed to various experimental conditions similar to those applied to honeybees. In the control group, which was not subjected to any stress, the gene expression level was 1.03. In contrast, the group exposed to liquid nitrogen cooling showed a significantly higher expression level of 5.61, indicating substantial molecular changes and responses. This treatment induced a strong heat shock response and increased *HSP70* expression.

When exposed to thermal stress, the gene expression levels of HSP70 in *Camponotus xerxes* increased progressively with temperature, reaching 3.07-fold at 45°C, 3.67-fold at 50°C, and peaking at 7.69-fold at 55°C. These findings demonstrate that gene expression changes correlate with increasing environmental stress severity. The variation between experimental groups is detailed in Table 5.

The control group of *Camponotus xerxes* exhibited no significant changes in *HSP70* gene expression, as expected, since it was not subjected to thermal or chemical stress. This confirms the stability of cellular functions under normal conditions.

In contrast, the liquid nitrogen-treated group displayed a significant increase in *HSP70* expression. This substantial difference compared to the control group suggests that chemical stress resulting from extreme cooling triggers a robust molecular defence response to protect cells from sudden environmental stress.

This substantial difference compared to the control group suggests that chemical stress from extreme cooling triggers a robust molecular defence response. Although not insect-specific, Hu *et al*. (2022) confirmed that chemical and physical stressors significantly enhance HSP70 expression across various biological systems, emphasising its conserved role in cellular protection mechanisms.

Upon exposure to moderate heat stress (45–50°C), *HSP70* gene expression levels increased compared to the control group but remained lower than in the liquid nitrogen-treated group (as shown in Table 5). This suggests a balanced cellular response to moderate thermal stress, demonstrating the ant's ability to activate protective pathways at non-lethal temperatures. These findings align with those of Xu *et al*. (2000), who reported that moderate thermal stress induces a moderate heat shock protein response.

At 55°C, *HSP70* expression was at its highest level (7.69), indicating an enhanced cellular protection response and reflecting a maximum adaptation mechanism to extreme thermal stress. This suggests that *Camponotus xerxes* exhibits remarkable thermotolerance, increasing *HSP70* production to stabilize cellular proteins and prevent degradation as part of its survival strategies.

This study highlights that social insects possess strong regulatory mechanisms for HSP70 gene expression, enabling them to endure extreme environmental stress. Heidari *et al*. (2024) similarly reported that desert-dwelling insects exhibit very high expression levels of heat shock proteins when exposed to extreme temperatures (>50°C), supporting our findings.

Relative estimation of *HSP70* gene expression in *Musca domestica*

The housefly (*Musca domestica*) is a widespread insect capable of surviving in diverse environments, ranging from hot to temperate climates, which contributes to its global distribution. Its adaptability to environmental changes is a key factor in its survival.

The results presented in Table 6, where houseflies (*Musca domestica*) were exposed to the same experimental conditions as ants and honeybees, indicate that the molecular response of *HSP70* gene expression differed from that observed in the other two insect species. This variation is due to the housefly's lifestyle and behavioral patterns, which revolve around food searching, reproduction, and risk avoidance—behaviors directly influenced by environmental conditions. Furthermore, houseflies can tolerate a wide range of environmental stressors.

The recorded HSP70 gene expression levels in *Musca domestica* varied across treatment conditions. In the control group, the expression level was 1.01, indicating baseline activity. Exposure to liquid nitrogen resulted in a notable increase to 4.94. Under heat stress, expres-

Table 6. Showing the *HSP70* gene expression levels in *Musca domestica* under different thermal conditions.

Musca domestica				
Groups	**Means Ct of House**	**Means Ct of HSP70**	**ΔCt (Means Ct of (HSP70**	**Fold of gene expression**
Control (No exposure to stress)	19.62	36.47	16.85	1.01
Nitrogen Exposed to liquid nitrogen, −96°C)	17.92	32.53	14.61	4.94
45°C (Moderate heat stress)	18.16	34.10	15.94	2.04
50°C (High heat stress)	18.00	34.05	16.06	1.91
55°C (Extreme heat stress)	19.62	33.29	14.38	5.62

Ct -Cycle threshold

sion levels were 2.04 at 45°C, slightly lower at 1.91 at 50°C, and peaked at 5.62 at 55°C, demonstrating a strong response to extreme thermal stress.

These findings highlight the impact of environmental stress on heat shock protein expression and its molecular response to increasing stress intensity (Table 6).

Under normal conditions, the control group of Musca domestica exhibited a stable level of HSP70 gene expression, enabling it to maintain essential biological functions. This stability is attributed to the absence of thermal or chemical stress.

However, in the liquid nitrogen-treated group, the houseflies exhibited a significant increase in *HSP70* expression. This response indicates a defensive mechanism to protect cells and proteins from misfolding and damage caused by rapid temperature shifts, which can disrupt protein function.

Such acute stress also affects mitochondrial activity, increasing the production of reactive oxygen species (ROS) and triggering cellular protective pathways, as confirmed by Tang *et al*. (2012), who demonstrated that stress-induced HSP70 in *Musca domestica* plays a functionally significant role in the insect's immune system.

Exposure to moderate heat stress (45–50°C) led to a slight increase in *HSP70* expression compared to the control group, though not as pronounced as in the liquid nitrogen-treated group. This suggests a balanced molecular response, reflecting the housefly's ability to adapt to mild environmental fluctuations. Houseflies are solitary insects with high environmental adaptability, which explains why their *HSP70* response to moderate heat stress is less dramatic than that of social insects.

The role of HSP70 in preventing protein damage and repairing heat-induced cellular damage aligns with findings by Tian *et al*. (2018), who reported that moderate heat stress in *Musca domestica* induces heat shock protein expression without excessive energy depletion, reflecting a balanced and adaptive stress response in solitary insects.

When the houseflies were exposed to 55°C, they exhibited the highest *HSP70* expression level (5.62), indicating a maximum protective response to extreme thermal stress. Compared to the liquid nitrogen-treated group, the expression difference was statistically significant, highlighting the insect's strong reaction to severe environmental challenges.

Extreme heat stress at 55°C affects mitochondrial membrane stability, disrupting energy production. If stress exceeds cellular recovery capacity, energy depletion may occur, as observed in studies by Colinet *et al*. (2016) on *Drosophila melanogaster* and related species. High temperatures also impair neurotransmission, reducing vital behaviors like predator evasion and foraging efficiency.*HSP70* plays a crucial role in protecting the central nervous system, as confirmed by research showing that heat stress induces gene responses that help preserve neural function (King & MacRae, 2015). These results align with the fly's molecular response observed at 45°C.

The present findings on the induction of HSP70 gene expression under thermal and chemical stress corroborate the established role of heat shock proteins in insect stress tolerance (Meng *et al*., 2022; Zhou *et al*., 2020; Tang *et al*., 2012). This study advances current knowledge in several important ways.

First, unlike many prior studies that focus on a single species or a narrow range of stressors, our research simultaneously compares three ecologically and behaviorally distinct insect species: Apis mellifera, Camponotus xerxes, and Musca domestica. This comparative approach reveals species-specific differences in HSP70 expression patterns, underscoring the varied adaptive strategies shaped by their unique ecological niches.

Second, by including both extreme heat (up to 55°C) and cryogenic stress (exposure to liquid nitrogen), the study explores molecular responses across a broader stress spectrum than most previous works, which typically focus on heat stress alone. This dual-stressor design provides a more comprehensive understanding of how insects cope with fluctuating environmental extremes, which is highly relevant under climate change scenarios.

Third, the current use of precise quantitative real-time PCR with detailed Ct value analysis provides robust, quantifiable insights into gene regulation dynamics, adding depth to the qualitative or semi-quantitative data

commonly found in earlier literature.

Together, these aspects make our study a significant contribution by elucidating both common and unique molecular defense mechanisms across insect species, thereby enhancing predictive models of insect resilience and informing conservation and pest management strategies in changing environments.

The study highlights that the housefly (*Musca domestica*) exhibited a distinct HSP70 gene expression pattern compared to the social insects—the honeybee (*Apis mellifera*) and the ant (*Camponotus xerxes*)—when exposed to environmental stressors. While all three species showed increased HSP70 expression under heat and cold stress, *Musca domestica* displayed a non -linear response, with moderate gene expression at 45° C (2.04-fold) and 50°C (1.91-fold), and a sharp increase at 55°C (5.62-fold) and under liquid nitrogen exposure (4.94-fold).

This contrasts with *A. mellifera* and *C. xerxes*, which exhibited progressive and consistent increases in gene expression as temperature stress intensified, peaking at 6.09-fold and 7.69-fold, respectively, at 55 °C. These findings suggest that houseflies possess a robust, yet threshold-based, protective mechanism that activates strongly only under extreme thermal stress, rather than gradually responding to increasing temperatures, as social insects do.

Despite this strong late response, our data indicate that excessive heat exposure (55°C) can compromise *M. domestica*'s cellular systems, potentially impairing mitochondrial integrity and neural function, as supported by literature linking extreme heat to oxidative stress and disrupted neurotransmission (Colinet *et al*., 2016; King & MacRae, 2015).

These results emphasise that while solitary insects, such as Musca domestica, are highly adaptable, their molecular defence mechanisms may only fully activate at high stress thresholds—unlike social insects, which deploy protective responses more gradually and consistently to maintain colony stability and function.

Conclusion

In the present study, the gene expression of heat shock protein HSP70 was evaluated in three insect species: honeybee (Apis mellifera), ant (Camponotus xerxes), and housefly (Musca domestica), each with differing ecological behaviours and stress tolerance capacities. Using RT-PCR, rhe study accurately quantified changes in HSP70 expression under various environmental stressors, including exposure to elevated temperatures (45°C, 50°C, 55°C) and liquid nitrogen cooling.The results revealed that HSP70 expression increased significantly in all species under extreme thermal and cold stress, with the highest induction observed at 55°C and after nitrogen exposure. Social insects (*A. mellifera* and *C. xerxes*) demonstrated a more progressive and consistent increase in gene expression with rising temperature, whereas the housefly (*M. domestica*) showed a non-linear pattern, with marked expression only under extreme conditions, suggesting species-specific molecular strategies for stress adaptation.This study employed a comparative approach, examining both heat- and cold-induced gene responses across three taxonomically and behaviorally distinct insects using standardised molecular tools. The provides a broader understanding of adaptive gene regulation in insects and the versatile role of HSP70 as a key molecular chaperone in cellular protection. Future studies should investigate the long-term physiological and behavioural effects of repeated thermal stress, as well as the interactions between HSP70 expression and other molecular stress pathways, to better predict insect resilience under climate change scenarios.

Conflict of interest

The authors declare that they have no conflict of interest.

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