

Thermal stress induces upregulation of *l(2)efl* in eggs of *Apis mellifera jemenitica* and *Apis mellifera carnica* (Hymenoptera, Apidae)

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Abstract

Apis mellifera subspecies exhibit strong adaptation to their respective natural habitats; however, they display varying responses to thermal stress. In the present study, we examined the expression of lethal(2)-essential-for-life-like (*l(2)efl*) gene variants (724449, 724231, 410087, 724405, 724488, 724274, and 724367), as well as histone-lysine N-methyltransferase trithorax (*trx*) and polycomb protein *Su(z)12* mRNA, in the eggs of two honeybee subspecies that exhibit clear differences in their thermal adaptation. The results revealed significantly higher expression levels of *l2efl* variants: 724449, 724231, 410087, 724405, 724488, 724274, and 724367), polycomb protein *Su(z)12* mRNA (ID: 409170), and histone-lysine N-methyltransferase (*trx*) (ID: 408716) in the eggs exposed to thermal stress compared to untreated controls in both *A. mellifera* subspecies. The main fold change between treated and control eggs reached approximately 200-fold for *l2efl*-724367, 100-fold for *l2efl*-724274, 40-fold for *l2efl*-724488, suggesting a robust heat-shock-like mechanism in response to thermal stress in early embryos of both subspecies. The results also showed distinct changes in *l2efl* and *trx* expression levels in both subspecies. While *A. m. carnica* exhibited higher expression of certain *l2efl* variants and *trx*, *A. m. jemenitica* showed stronger activation of other *l2efl* members and *Su(z)12*. The expression of *Su(z)12* increased 86-fold more in *A. m. jemenitica* than in *A. m. carnica*. This might reflect different adaptive strategies in both subspecies. These findings suggest that thermal exposure in *A. mellifera* embryos not only triggers classical stress-response genes but also modulates epigenetic pathways that could contribute to transgenerational thermotolerance.

Key Words

Gene expression, honeybee, HSP, *l(2)efl*, thermal adaptation

Introduction

Apis mellifera is naturally distributed in highly variable habitats with more than 30 distinctive subspecies (Ruttner 1988; Ilyasov et al. 2020). In these environments, *A. mellifera* typically builds their nests in cavities, where worker bees keep the colony's internal temperature within a narrow range (33.5–36.5 °C). This intensive form of social homeostasis is exceptionally unique among insects and is essential for egg laying, brood survival and normal development in *A. mellifera* colonies (Southwick and Heldmaier 1987; Jones et al. 2005). To achieve this, *A. mellifera* workers rely on a combination

of physiological and behavioral mechanisms including intentional warming, fanning, and hive-evaporative cooling to maintain optimal brood temperature (Lindauer 1955; Heinrich 1981; Seeley and Heinrich 1981; Southwick 1987; Stabentheiner et al. 2010). Queens may also modify their egg-laying dynamics in response to extreme ambient temperature fluctuations (Ruttner 1988; Alqarni 1995; Amiri et al. 2020), particularly when such conditions coincide with reduced nectar and pollen availability.

Under prolonged extreme temperature regimes, exotic *A. mellifera* colonies may fail to withstand these unfavorable conditions, resulting in declining colony population density, absconding behavior, or colony death (Alqarni

1995; Ali 2011; Alattal and Alghamdi 2015; Al-Ghamdi et al. 2017). In contrast, native *A. mellifera* subspecies exhibit distinctive adaptations that allow them to better accommodate the environmental demands of their natural habitats (Ruttner 1988; Alattal and Alghamdi 2015). Beyond social homeostasis behaviors, adapted *A. mellifera* subspecies evolved inherited adaptive morphological, physiological and structural traits across colony members. Consequently, adult *Apis mellifera* as eurytherm, exhibit superior performance in their native habitats, compared to introduced *A. mellifera* subspecies (Ruttner 1988; Alattal and Alghamdi 2015). On Contrast, the brood is stenotherm and requires a narrower temperature range for proper development (Seeley and Heinrich 1981; Kleinhenz et al. 2003; Petz et al. 2004; Tautz et al. 2004; Stabentheiner et al. 2010).

Adult *A. mellifera* forage across a wide temperature range 10–40 °C with an optimal range of 22–25 °C. However, ideal brood development occurs between 33–36 °C, and deviation as small as 1 °C can severely affect brood survival and emergence, larval growth, pupal morphology, and behavioral and neural development (Petz et al. 2004; Jones et al. 2005; Becher et al. 2009; Medina et al. 2018; McAfee et al. 2020). Within the brood chamber, eggs are particularly vulnerable to elevated temperature, which can cause mortality or desiccation. *A. mellifera* subspecies exhibit notable variation in egg size and oviposition rates, in response to environmental conditions (Ruttner 1988; and Amiri et al. 2020). Research indicates that *A. mellifera* queens in smaller colonies tend to produce eggs with larger size, whereas those in larger colonies lay smaller eggs, while this variation appears non-adaptive, and is more likely a form of phenotypic plasticity (Han et al. 2022), queen adjustment of egg size and fecundity under environmental stressors such as drought and elevated temperatures is an adaptation that increases survival rates and reproduction success under harsh conditions (Fox and Czesak 2000; Amiri et al. 2020). Moreover, differences in egg size among *A. mellifera* subspecies have been linked to variation in egg water content under various thermal regimes (Al-Ghamdi et al. 2014), suggesting that these traits play a critical role in thermal adaptation.

A. m. carnica and *A. m. jemenitica* are two honeybee subspecies showing clear thermal adaptation in their respective natural habitats. *A. m. carnica* is a temperate-zone subspecies, showing adaptive traits to long, cold winters including larger size, darker color, efficient overwintering, distinct colony dynamics, cluster and winter bees etc.) (Ruttner 1988). In contrast, *A. m. jemenitica* is a subtropical subspecies, well adapted to extreme high temperature and drought conditions (Ruttner 1988).

Under desert conditions of Saudi Arabia, where summer temperatures exceed 42 °C, most *A. m. carnica* colonies performs poorly compared to *A. m. jemenitica*, with very high colony loses (~84%) within the first year of introduction (Alattal and Alghamdi 2015). Numerous studies have highlighted the thermal adaptation of *A. m. jemenitica*. Key adaptive traits include smaller body

size, lighter pigmentation, smaller nest size, rapid colony growth, earlier foraging activity, and increased expression of heat-shock proteins (HSPs) and the lethal(2) essential-for-life-like (*l(2)efl*) gene (Ruttner 1988; Alattal 2015; Alattal and Alghamdi 2022, 2023, 2024). At the brood level, structural, physiological and behavioral traits of the brood and brood nest contributed to colony-level thermal adaptation (Ruttner 1988). Compared with *A. m. carnica*, *A. m. jemenitica* displays smaller brood cell size, optimized oviposition rates, shorter post-capping periods, and smaller eggs with higher moisture content, all of which enhance survival under extreme thermal stress (Gadbin et al. 1979; Ruttner 1988; Adgaba et al. 2016).

On the molecular level, *A. m. jemenitica* foragers demonstrate substantially higher expression of heat-shock proteins (HSPs; hsp70ab, hsc70cb, hsp83, hsp90, hsp10, hsp28) compared with *A. m. carnica* under the desert summer conditions of Saudi Arabia (Al-Ghamdi and Alattal 2023). Similarly, day-long real-time expression levels of *l(2)efl* genes were reported to be higher in *A. m. jemenitica* than in *A. m. carnica* (Al-Ghamdi and Alattal 2023), suggesting enhanced basal and phenotypic thermal tolerance in the former. Fold changes in *l(2)efl* expression reached approximately 100× higher in *A. m. jemenitica* than in *A. m. carnica* under thermal stress (Alattal and Alghamdi 2024; Al-Ghamdi and Alattal 2024). HSPs, including *l(2)efl*, can also be upregulated in immature stages of *A. mellifera* in response to environmental stressors (Seeley and Heinrich 1981; Sahebzadeh and Lau 2017). In eggs, HSPs are expressed constitutively at low levels but can be further induced under thermal stress, with expression variations among insect species (Saravanakumar et al. 2008; Wang et al. 2016; Jia et al. 2020). While numerous studies have investigated HSP expression patterns in adults and larvae, very little information exists for eggs.

The *lethal(2)-essential-for-life-like* (*l(2)efl*) gene belongs to the HSP20 family and is critical for thermal adaptation and protein homeostasis in *A. mellifera* (Alattal and Alghamdi 2024). Beyond HSPs, other genes are essential for successful embryonic development and survival. *Suz12* is a key component of the Polycomb Repressive Complex 2 (PRC2), and its repression can lead to early embryonic lethality (Pasini et al. 2007). Histone-lysine N-methyltransferases (*trx*) comprise a family of enzymes involved in developmental processes such as proper cell differentiation, maintenance of cell integrity, and early regulation of homeotic genes expressed in the posterior region of the embryo (UniProtKB, accessed 22 October 2025). In the present study, we investigate whether acute heat stress induces upregulation of *l(2)efl* transcription, *Suz12* and *trx*, acting as a protective strategy that reflects an early molecular heat-stress response during embryonic development in *Apis mellifera* eggs. We further hypothesize that eggs from heat-tolerant *A. mellifera* lineages (*A. m. jemenitica*) may exhibit a different *l(2)efl* transcriptional response compared to eggs from heat-sensitive lineages (*A. m. carnica*).

Methodology

Study site and honeybee colonies preparation

Apis mellifera colonies were established at the Riyadh Educational Apiary, King Saud University (24°73'80"N, 46°62'09"E). Riyadh represents a typical desert climate, characterized by extreme heat (summer temperatures exceeding 46 °C) and low annual precipitation (~100 mm), under which only *A. m. jemenitica* can survive, while exotic subspecies such as *A. m. carnica* often experience high colony losses. Eight *A. m. jemenitica* colonies were established from a previously certified in-house population (Bee Research Unit, King Saud University). Another eight *A. m. carnica* colonies were established using pure-bred Carniolan queens (LOKACIJA, Slovenia) introduced into eight queenless package bees (1.5 kg each). Each package was headed by a single *A. m. carnica* queen. Carniolan queens were introduced into the packages early Spring (Average Maximum temperatures ~22–26), at this time the colonies usually start population increase and brood rearing in under the ecosystem of Riyadh. Three months after colony establishment, the subspecies affiliation of each colony was confirmed using standard morphometric methods (body dimensions, pigmentation, and wing venation; Bouga et al. 2011; Meixner et al. 2013) and reference morphological data obtained from the Oberursel Bee Research Institute (Frankfurt, Germany). Established colonies (n = 16, 8 per subspecies) consisted of 7–8 frames covered with adult bees, including 3–4 brood frames, and were maintained under identical conditions throughout the study.

Eggs sampling

Egg sampling was conducted in April. From each colony, thirty eggs (~8 h old) were collected. For this purpose, one empty, fully molded frame was inserted into the center of the brood frames of each colony. After 24 h, the inserted frames were removed, cleaned of any existing eggs, and returned to the colonies. Then, thirty newly laid eggs (~8 h old) were collected from each frame using a fine brush and placed in a 2 ml Eppendorf tube. In total, 240 eggs were collected from *A. m. carnica* colonies and 240 eggs from *A. m. jemenitica*. To investigate gene expression in both subspecies, eggs from each subspecies were pooled and randomly distributed into two Eppendorf tubes (120 eggs per tube). Thus, there were four tubes in total: two for control and two for heat treatment. Control samples of each subspecies were immediately dipped in liquid nitrogen and stored at –80 °C. Eggs assigned for heat treatment were placed in a 10 ml glass Petri dish and incubated at 42 ± 1 °C and 75 ± 0.5% relative humidity for one hour (Memert HCP 105, Germany). After heat treatment, eggs were transferred to new tubes, immediately frozen in liquid nitrogen, and stored at –80 °C for RNA extraction.

RNA extraction and cDNA synthesis

Eggs from each sample were macerated, and total RNA was extracted using the TRIzol™ Plus RNA Purification Kit (Invitrogen, California, USA) following the manufacturer's protocol. The extracted RNA was further purified using a Qiagen RNeasy column (Qiagen, Germantown, TN, USA). First-strand cDNA was then synthesized from the purified RNA using the SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen, California, USA). The synthesized cDNA served as the template for subsequent real-time PCR analysis.

Primer design and real-time PCR

Sequences of the lethal(2)-essential-for-life-like (*l(2)efl*) genes (ID: 724405, 724488, 724274), histone-lysine N-methyltransferase trithorax (*trx*; ID: 408716), and polycomb protein Su(z)12 (ID: 409170) from *A. mellifera* were obtained from the Honeybee Genome Consortium (NCBI Database). Primers were designed using Geneious® Prime v.2019.2.3 (<https://www.geneious.com>, Biomatters Ltd., Newark, NJ, USA) (Table 1). qPCR assays were performed using SYBR® GREEN Master Mix (Applied Biosystems, Carlsbad, CA, USA) on an Applied Biosystems 7500 Real-Time PCR system. Each 25 µl reaction contained 13 µl of 2X master mix, 2 µl of each primer (2 pmol), 2 µl of cDNA template, and 7 µl of nuclease-free water. β-actin was used as an endogenous control. All reactions were run in triplicate under the following cycling conditions: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 57 °C for 30 s, and 72 °C for 10 s, with a final step of 95 °C for 20s.

Statistical analysis

Relative gene expression and fold-change analyses were calculated using qPCR Ct values according to the sampling plan. β-actin Ct values were used as the endogenous reference for calculating relative expression levels (relative expression = $2^{-(Ct_{\text{actin}} - Ct_{\text{reference}})}$). To analyze fold changes, average Ct values of untreated *A. m. carnica* sample were used as calibrator (fold change = $2^{-(Ct_{\text{reference}} - Ct_{\text{gene}})}$) (Schmittgen and Livak 2008). Significant differences among study groups were evaluated by sample unpaired t test (p < 0.05) using the Statistical Analysis System software suite (SAS Institute: <https://www.sas.com>). Figures were prepared using GraphPad Prism 8.0.1.

Results

Results revealed markedly higher expression levels of *l2efl* (Variants: 724449, 724231, 410087, 724405, 724488, 724274, and 724367), polycomb protein Su(z)12 mRNA (ID: 409170), and histone-lysine N-methyltransferase (*trx*) (ID: 408716) in thermally treated eggs

Table 1. Gene-ID (NCBI) for *Apis mellifera* *lethal(2)-essential-for-life-like* (*l(2)efl*) genes, *A. mellifera* histone-lysine N-Methyl Transferase (*trx*) and (*A. mellifera*) polycomb protein *Su(z)12*, mRNA with gene location, length and primer sequences designed and used in this study.

Gene ID/Gene Name	Location (LG2)/length (nt) Primers' position	Primers
<i>l(2)efl</i> ID:724405	(LG2):4,831,304...4,832,577/1274 (F:4831846-4831866; R:4831983-4831964)	F-TGCGACATCGATCAAGCGTCC R-TTGCATCGCACGGTTTCC
<i>l(2)efl</i> ID:724488	(LG2):4,837,466...4,838,343/878 (F:4837783-4837803; R:4837937-4837918)	F-ACCTTGGGGTGAACCTTCTGCG R-TCCCCTCGACGACAACACAC
<i>l(2)efl</i> ID:410087	(LG2):4,837,466...4,838,343/878 (F:4825423-4825446; R:4825745-4825723)	F-ACCGTGATCATCTGCCTTGTCTTC R-TCGAGTGATTCTTGACGTGCAGC
<i>l(2)efl</i> ID:724274	(LG2):4,823,146...4,824,181/1063 (F:4823390-4823413; R:4823489-4823467)	F-TCACCGAGCCGATTGGAGTTATGT R-AACTGCCTCTGTACCACGAAAC
<i>l(2)efl</i> ID:724231	(LG2):4,823,146...4,824,181/1063 (F:4821284-4821303; R:4821392-4821369)	F-TGCGAAAACAGCGACGAGGT R-CCCTTTGACCAGCTTCTGGACTT
<i>l(2)efl</i> ID:724449	(LG2):4,823,146...4,824,181/1063 (F:4834305-4834327; R:4834404-4834380)	F-TCGACCACATAAACGCGGCAGAA R-TTGAACAATAGATGCACCGCAGCA
<i>l(2)efl</i> ID:724367	(LG2):4,823,146...4,824,181/1063 (F:4829515-4829534; R:4829622-4829602)	F-CGAGTGTGTCTCCGATGGC R-GAGTGGTTTCCCCGTGAAC
<i>A. mellifera</i> HMT (<i>trx</i>) ID: 408716	(LG2):4633633...4650053, complement (F:4638036-4638611; R:4637736-4637760)	F-TGCAGCTAGATTCATTAATCATTAT R-CATGGAATCTTGATATCTCGAAAG
<i>A. mellifera</i> polycomb protein <i>Su(z)12</i> , mRNA ID: 409170	(LG10)11912175...11918682/6507 (F:11916106-11916130; R:11916288-11916264)	F-TCTGAAGGAGAAAATGATCCAAAAT R-TTAGTTTCTAGAAACATTGGCAA

compared to untreated controls in both *A. mellifera* subspecies (Fig. 1). The main fold change between treated and control eggs reached approximately 200-fold for *l2efl-724367* (Fig. 1), 100-fold for *l2efl-724274*, 70-fold for *Su(z)12* mRNA, 40-fold for *l2efl-724488*. Among the *l2efl* variants, *l2efl-724488*, *l2efl-724274*, *l2efl-724367*, and polycomb protein *Su(z)12* mRNA (ID: 409170) exhibited much higher fold changes in expression than *l2efl-724449*, *l2efl-724231*, *l2efl-410087*, *l2efl-724405*, and *trx* (Fig. 1). Comparative analysis between subspecies showed that fold changes in *l2efl-724449*, *724231*, *410087*, and *trx* were 3–38 × higher in treated *A. m. carnica* eggs than in *A. m. jemenitica* (Table 2), nonetheless, changes in expression levels in these variants were relatively small (Fig. 1). Conversely, fold changes in *l2efl-724488*, *724274*, *724367*, and *724405* were 1.29–3.80 × higher in *A. m. jemenitica* than in *A. m. carnica* (Table 2). Notably, *Su(z)12* (ID 409170) expression was 86 × higher in treated eggs of *A. m. jemenitica* compared with *A. m. carnica* (Table 2). In untreated eggs, *l2efl-724367*,

724272, *724449*, *724231*, and *724405* showed slightly higher expression in *A. m. carnica* than in *A. m. jemenitica* (1–3 × difference; Table 3), whereas *l2efl-410087*, *410088*, and *trx* were higher in *A. m. jemenitica* (1.10–2.43 × more). Additionally, *Su(z)12* (ID 409170) expression in untreated eggs was 1.15 × higher in *A. m. jemenitica* compared with *A. m. carnica*.

Discussion

Thermal treatment induced pronounced transcriptional changes in eggs of both *A. m. carnica* and *A. m. jemenitica*, as demonstrated by the significant upregulation of *l2efl*, *Su(z)12*, and *trx* genes. The markedly higher expression of *l2efl* variants, particularly *l2efl-724367* (up to 200-fold), highlights the activation of heat-responsive molecular chaperones likely functioning to protect embryonic proteins from thermal denaturation. The *l2efl* gene family, which encodes small heat shock-like

Table 2. Comparison of relative expression levels (M ± SD), fold changes and statistical analysis parameters (P-value, R², F test for variances) of *l2efl* gene variants, *trx* and polycomb protein *Su(z)12* in treated eggs (thermal stress) of *A. m. jemenitica* and *A. m. carnica*.

Gene ID	Relative expression (m ± sd)		Fold change	T test P value	R ²	F test (variances)
	<i>A. m. jemenitica</i>	<i>A. m. carnica</i>				
<i>l2efl-724367</i>	202.9313	90.41185	2.25	<0.0001	0.9982	0.3216
<i>l2efl-724274</i>	100.7011	53.77075	1.87	<0.0001	0.9948	0.7106
<i>l2efl-724449</i>	0.409111	13.2412	0.30	<0.0001	0.9914	0.1164
<i>l2efl-724231</i>	1.311281	5.24662	0.25	<0.0001	0.9907	0.0170
<i>l2efl-410087</i>	0.37506	14.32919	0.03	<0.0001	0.9980	0.3046
<i>l2efl-724488</i>	40.38236	31.24045	1.29	0.0005	0.9626	0.3040
<i>l2efl-724405</i>	4.911232	1.292462	3.80	<0.0001	0.9886	0.1658
(<i>trx</i>) transcript variant X4-408716	2.530234	3.998426	0.63	<0.0001	0.9911	0.5718
Polycomb protein <i>Su(z)12</i> -409170	68.33552	0.786546	86.88	<0.0001	0.9991	0.0090

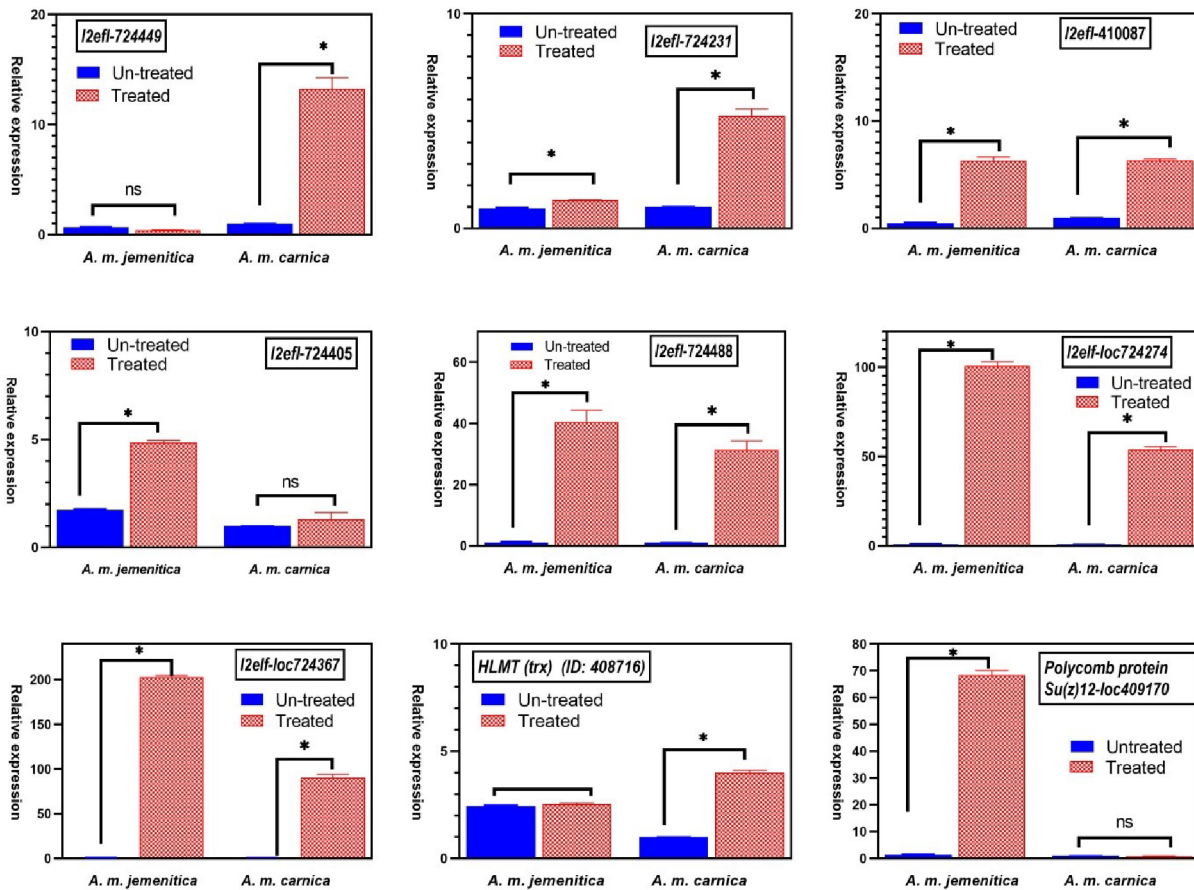


Figure 1. Fold changes of (*mRNA*) relative expression levels for *Apis mellifera* lethal(2)-essential-for-life-like (*l2elf*) genes (ID: 724449; 724231; 410087; 724405; 724488; 724274); *Apis mellifera* histone-lysine N-MT (*trx*) transcript variant X4 (ID: 408716), and *Apis mellifera* polycomb protein *Su(z)12* (ID: 409170) in *A. m. jemenitica* (local) and *A. m. carnica* (carnica) at two thermogeographical regions (Riyadh: R and Baha: B) and the three foraging times (7:00 a.m.: after ≈1 h of foraging; 12:00 p.m.; and 5:00 p.m. (≈1 h before sunset)). Δ ct was calculated by using actin as endogenous control, and the fold change (fold change = $2^{\text{ct}}(\text{reference-treatment})$) was calculated using the relative expression in *A. m. carnica* in the Baha region as reference (Calibrator). Significant fold change difference was determined based on average gene expression of each group using a three-way ANOVA analysis followed by Multiple comparisons based Bonferroni post hoc test, $p < 0.05$. (*) = significant variation and (ns) = non-significant variation.

Table 3. Comparison of relative expression levels ($M \pm SD$), fold changes and statistical analysis parameters (P-value, R^2 , F test for variances) of *l2elf* gene variants, *trx* and polycomb protein *Su(z)12* in un-treated eggs of *A. m. jemenitica* and *A. m. carnica*.

Gene ID	Relative expression ($m \pm sd$)		Fold change	T test P value	R^2	F test (variances)
	<i>A. m. jemenitica</i>	<i>A. m. carnica</i>				
l2elf-724367	0.581807	1	0.58	<0.0001	0.9953	0.2793
l2elf-724274	0.992929	1	0.99	0.7431	0.02992	0.8142
l2elf-724449	0.665252	1	0.67	<0.0001	0.9918	0.9866
l2elf-724231	0.926071	1	0.93	0.0327	0.7200	0.3895
l2elf-410087	1.101329	1	1.10	<0.0001	0.9970	0.1967
l2elf-724488	1.728426	1	1.73	0.5343	0.1034	0.6353
l2elf-724405	0.334701	1	0.34	<0.0001	0.9931	0.1118
(<i>trx</i>) transcript variant X4-408716	2.427028	1	2.43	<0.0001	0.9981	0.0778
Polycomb protein <i>Su(z)12</i> -409170	1.144506	1	1.15	0.0012	0.9429	0.9952

proteins, has previously been associated with enhanced thermotolerance and cellular protection in *A. mellifera* foragers under thermal stress (Alattal and Alghamdi 2023). These findings indicate that early embryos of both subspecies possess a robust heat-shock-like mechanism rapidly triggered by thermal stress.

The pronounced upregulation of *l2elf*, *Su(z)12*, and *trx* in thermally treated eggs suggests a strong molecular response involving both stress-related and epigenetic regulatory pathways. *Su(z)12* and *trx* are key epigenetic regulators belonging to the Polycomb and Trithorax group complexes, which maintain chromatin structure

and regulate transcriptional memory of developmental genes. Upregulation of Su(z)12 supports the involvement of Polycomb-mediated repression in response to thermal stress, consistent with its recognized role in insect and mammalian development (Cao et al. 2002; Alattal et al. 2024). This may represent an adaptive mechanism in which thermal stress induces epigenetic reprogramming to stabilize gene expression patterns essential for normal embryogenesis under fluctuating temperatures. Epigenetic upregulation of *l2efl* was also reported in *A. mellifera* foragers exposed to heat stress, suggesting that H3K4/H3K27 methylation changes underpin *l2efl* activation under thermal challenge (Alattal and Alghamdi 2023, 2024).

The observation that Su(z)12 expression increased 86-fold more in *A. m. jemenitica* than in *A. m. carnica* indicates that epigenetic modulation may constitute a more pronounced adaptive response in the desert-adapted *A. m. jemenitica*. Subspecies-specific differences in *l2efl* and *trx* expression further emphasize divergent thermotolerance strategies: while *A. m. carnica* exhibited higher expression of certain *l2efl* variants and *trx*, *A. m. jemenitica* showed stronger activation of other *l2efl* members and Su(z)12. These differences likely reflect distinct evolutionary adaptations to their native climatic conditions: *A. m. carnica*, adapted to cooler temperate zones, may rely more on protein-stabilizing mechanisms, whereas *A. m. jemenitica*, native to arid and hot regions, may depend more on epigenetic control for developmental plasticity under heat stress. These findings help explain the poor performance and low survival rates of *A. m. carnica* colonies compared to *A. m. jemenitica* under Saudi Arabian summer conditions, where temperatures exceed 42 °C (Alattal and Alghamdi 2015).

Overall, thermal exposure in *A. mellifera* embryos triggers classical stress-response genes while also modulating epigenetic pathways that may contribute to transgenerational thermotolerance.

Future studies integrating chromatin profiling, methylation analysis, and gene silencing could clarify how these transcriptional changes translate into long-term developmental and adaptive outcomes in thermally challenged honey bee populations.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, Y.Z.A. and A.A.A.; methodology, Y.Z.A.; software, Y.Z.A.; validation, Y.Z.A. and A.A.A.; formal analysis, Y.Z.A.; investigation, Y.Z.A.; resources, A.A.A.; data curation, Y.Z.A.; writing—original draft preparation, Y.Z.A.; writing—review and editing, Y.Z.A. and A.A.A.; visualization, Y.Z.A.; supervision, Y.Z.A. and A.A.A.; project administration, A.A.A.; funding acquisition A.A.A. All authors have read and agreed to the published version of the manuscript.

Artificial Intelligence (AI) use

Regarding the use of AI in the preparation of this manuscript, the authors declare the following:

References and some English Language editing

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Data Availability Statement

Data are available in tables and figures.

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