Citation: Abou-Shaara HF. Variations in heat shock proteins between different honey bees and bee taxa utilizing bioinformatics. U. Bee J. / U. Arı D. 24 (1): 38-52, DOI: 10.31467/uluaricilik.1390515

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

VARIATIONS IN HEAT SHOCK PROTEINS BETWEEN DIFFERENT HONEY BEES AND BEE TAXA UTILIZING BIOINFORMATICS

Biyoinformatik Kullanılarak Farklı Bal Arıları ve Arı Taksonları Arasında Isı Şoku Proteinlerindeki Varyasyonlar

Hossam F. ABOU-SHAARA

Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, 22516, EGYPT, ORCID No: 0000-0001-7208-6526, E-posta: hossam.farag@agr.dmu.edu.eg

Geliş Tarihi / Received: 14.11.2023 Kabul Tarihi / Accepted: 07.02.2024 DOI: 10.31467/uluaricilik.1390515

ABSTRACT

The changes in climate and exposure to heat stress are major concerns for agricultural communities as it affects pollinators like bees. Bees from different taxa play a crucial role in plant pollination, and their exposure to heat stress induces the expression of heat shock proteins (HSPs) to protect their cells. Several studies have analyzed the variations in HSPs expression levels and amino acid sequences. Databases for sequences of HSPs with different molecular weights are currently available. Variations in HSPs expression levels have been noted among individuals belonging to the same or different bee taxa exposed to heat stress. The properties of HSPs could help in understanding these variations. This study utilized bioinformatics and protein analysis tools to investigate the variations in sequences of heat shock proteins 60 (HSP60) and 83 (HSP83) in 18 bee taxa (15 from Family Apidae, 2 from Family Halictidae, and one from Megachilidae). The analysis showed some identical values to bees from genus *Apis* and *Bombus.* For HSP60, all bee taxa had high G content (587-602), followed by A (438-444), then C (389-404), and finally T (282-291). For HSP83, all bee taxa had high A content (730-759), followed by G (572-592), then C (406-419), and finally T (415-429). The conserved domains were highly identical in case of HSP60 versus HSP83. The motifs were from one or more protein families with variation among taxa. All proteins showed hydrophilic properties with variable isoelectric points. The study suggested an identical 3-D structure for proteins in all bee taxa. The role of the detected variations in affecting the response of HSPs to stress was discussed. This study paves the way for more investigations on HSPs and encourages the use of bioinformatics and protein analysis tools to explain any observable variations.

Key Words: Honey bees, Bioinformatics, Stress, Climate, HSP

ÖZ

İklimdeki değişiklikler ve tozlaştırıcıların ısı stresine maruz kalması, tarım topluluklarının en büyük endişeleri arasında yer alıyor. Farklı taksonlara ait arıların bitkilerin tozlaşmasında büyük rolü vardır. Arıların ısı stresine maruz kalması, vücut hücrelerini korumak için ısı şoku proteinlerinin (HSP'ler) ekspresyonuna neden olur. Birçok çalışma HSP'lerin ekspresyon seviyelerindeki farklılıkları araştırmış ve amino asit dizilerini analiz etmiştir. Şu anda, farklı molekül ağırlıklarına sahip HSP dizileri için veritabanları mevcuttur. Aynı arı taksonuna ait veya ısı stresine maruz kalan farklı taksonlara ait bireyler arasında HSP'lerin ekspresyon seviyelerindeki farklılıklar kaydedilmiştir. HSP'lerin özelliklerinin bu tür farklılıkların anlaşılmasına yardımcı olabileceği varsayılmaktadır. Bu çalışmada, 18 arı taksonunda (15'i Apidae familyasından, 2'si Halictidae familyasından ve bir Megachilidae'den). Amino asitlerin ve nükleotidlerin analizi, Apis ve Bombus cinsinden arılarla aynı değerleri gösterdi. Korunan alanlar, HSP60 durumunda HSP83'e göre oldukça özdeşti. Motifler, taksonlar arasında

çeşitlilik gösteren bir veya daha fazla protein ailesindendi. Tüm proteinler değişken izoelektrik noktalarla hidrofilik özellikler gösterdi. Çalışma, tüm arı taksonlarındaki proteinler için aynı 3 boyutlu yapıyı öne sürdü. Tespit edilen varyasyonların HSP'lerin strese tepkisini etkilemedeki rolü tartışıldı. Bu çalışma HSP'ler hakkında daha fazla araştırmanın önünü açıyor ve gözlemlenebilir varyasyonları açıklamak için biyoinformatik ve protein analiz araçlarının kullanımını teşvik ediyor.

Anahtar Kelimeler: Bal arıları, Biyoinformatik, Stres, İklim, HSP

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Bu çalışma, farklı arı taksonlarının HSP60 ve HSP83 arasındaki benzerliklerine/farklılıklarına ışık tutmayı amaçlamaktadır. Bu, arı türleri/alt türleri arasında HSP'lerin gen ekspresyon düzeylerindeki farklılıkların açıklanmasına yardımcı olabilir. Ayrıca bu çalışma, biyoinformatik kullanarak arıların HSP'lerini moleküler düzeyde daha ayrıntılı olarak incelemek için yollar sunmaktadır.

Gereçler ve yöntemler: Isı şoku proteinleri (HSP'ler), farklı arı taksonları için GenBank'tan tarandı. Son olarak bu çalışmada 18 takson ele alınmış olup bunların 15'i Apidae familyasına, 2'si Halictidae familyasına ve 1'i Megachilidae familyasına aittir. Proteinlerin moleküler ağırlıkları, moleküler ağırlık hesaplayıcı kullanılarak değerlendirildi. Daha sonra çalışmada iki protein türü dikkate alındı: HSP60 ve HSP83. Proteinlerin amino asit bileşimleri moleküler ağırlık hesaplayıcı kullanılarak değerlendirildi. Amino asit dizileri, çevrimiçi bir çeviri aracı kullanılarak yaklaşık nükleotid dizilerine çevrildi. Daha sonra, her bir nükleotid tipinin sayısı ve %G~C, dizi analiz aracı kullanılarak hesaplandı. Dizilerdeki korunan alanlar, ncbi.nlm.nih.gov/Structure adresindeki "korunan alanları tanımlama" aracı kullanılarak tanımlandı. Ayrıca çalışılan proteinlerdeki motifler MOTIF Search kullanılarak tarandı. Çalışılan arıların proteinleri arasındaki farklılıkları anlamak için bioinformatics.org'da bulunan protein analizi seçeneklerinden yararlanıldı. Çalışmada kullanılan proteinlerin en özdeş 3 boyutlu yapısı incelendi.

Bulgular: Farklı arı taksonlarının HSP60 için amino asit kompozisyonları bazı taksonlar dışında aynıydı. HSP83 için farklı arı taksonlarının amino asit kompozisyonlarındaki farklılıklar, bazı türler/alt türler arasında aynı kompozisyonları göstermiştir. HSP60 için tüm arı taksonları yüksek G içeriğine (587-602) sahipti, bunu A (438-444), ardından C (389-404) ve son olarak T (282-291) izledi. HSP83 için tüm arı taksonları yüksek A içeriğine (730-759), ardından G (572-592), ardından C (406-419) ve son olarak T (415-429) içeriğine sahipti. %G~C değeri %44.9-46.7 aralığındaydı. Dizilerdeki korunmuş alanlar, HSP60 için 23'ten 541'e kadar yüksek özdeş değerler gösterdi. Dizilerdeki korunan alanlar, HSP83 için bazı arı taksonlarında özdeşti. HSP60 ve HSP83'ün motifleri taksonlara dayalı bir veya daha fazla protein ailesine aitti. HSP60 ve HSP83'ün negatif GRAVY değerleri tespit edildi. Sonuçlar, çalışılan proteinlerin 3 boyutlu yapısının, çalışılan arı taksonlarında oldukça aynı olduğunu gösterdi.

Sonuç: Çalışma, özellikle Apis ve Bombus cinsi arılarda bazı özelliklerde aynı değerlerin varlığını göstermiştir. Bununla birlikte, HSP60 ve HSP83'teki farklılıklar, stres koşulları altında arı taksonları arasındaki tepki seviyelerindeki potansiyel farklılıklara bir açıklama sunmaktadır. Çalışma, yalnızca HSP'lerin ekspresyonunu incelemenin değil, aynı zamanda dizileri analiz etmeye devam etmenin ve proteinler üzerinde daha fazla analiz yapmanın önemini ortaya koyuyor.

INTRODUCTION

Pollination is very essential for agriculture worldwide (Winfree et al, 2011; Lautenbach et al, 2012). The pollination is achieved by many insect taxa, especially bees (Kearns & Inouye 1997, Heard 1999; Nicholls & Altieri 2013). Thus, any decline in pollinators poses a public concerns with worries related to food security (Kevan & Viana, 2003; Thomann et al, 2013). The genus *Apis* contains the most important plant pollinators and includes the managed honey bee, *Apis mellifera* (Aslan et al, 2016, Hung et al, 2018). There are about 33 subspecies of *A. mellifera* distributed geographically in many countries (Engel 1999, Ilyasov et al, 2020, Sheppard & Meixner 2003). In addition to *Apis*, there are different genera that contribute to pollination such as *Bombus*, *Melipona*, and various species of wild bees (Arretz & Macfarlane 1986, Bernauer et al, 2022, Hausmann et al, 2016, Whitehorn et al, 2013). One of the major threats to biodiversity in the future is the change in climate due to global warming

(Bellard et al, 2012, Easterling et al, 2000; Thomas et al, 2004, Sangle et al, 2015). Climate change is expected to affect distribution, activities, and physiology of insects (Abou-Shaara and Al-Khalaf, 2022, Abou-Shaara et al, 2022David et al, 2005, Du et al, 2007, Karl et al, 2011, Pettis et al, 2016, Sales et al, 2018), and harming the managed bee colonies (Abou-Shaara, 2016, Le Conte & Navajas, 2008, Reddy et al, 2012).

Bees, as major pollinators, are affected by any changes in the ambient temperature (ectothermic organisms). Such changes can affect their development and activities (Abou-Shaara et al, 2017, Alqarni, 2006, Blažytė-Čereškienė et al, 2010, Stabentheiner et al, 2010, Tautz et al, 2003). In the European context, the *Apis mellifera mellifera* subspecies, commonly known as the dark European honey bee, stands out as the subspecies most well-suited to endure extremely low temperatures, reaching about -30˚C. Additionally, this subspecies undergoes an extended wintering period lasting around 6 months, during which the bees do not engage in flight. However, the resilience of these cold-hardy bees is being notably challenged by the effects of global warming (Ilyasov et al, 2020). The variations in heat resistance have been observed for different bee taxa such as honey bees, *Apis* spp., and bumble bees, *Bombus* spp. (Abou-Shaara et al, 2012, Martinet et al, 2015, Oyen et al, 2016, Zambra et al, 2020). The exposure of bees to several stressors such as pathogens, chemicals, and heat stress induces the expression of heat shock proteins (HSPs) (Candido 2001, Elekonich, 2009, Koo et al, 2015, Severson et al, 1990). Thus, HSPs can serve as biomarkers for cellular stress (Gibney et al, 2001, Nazir et al, 2003); especially, they are highly expressed cellular proteins across divergent organisms (Csermely et al, 1998). The expression of HSPs has been linked with longevity, fecundity, inhibition of cell death, stress tolerance, and mitochondrial damage suppression (Hoffmann et al, 2003, Feder & Hofmann 1999, Mosser et al, 1997, Qi et al, 2011, Vermeulen & Loeschcke, 2007). The molecular weight is generally used to classify HSPs into categories such as HSP60, HSP70, and HSP90 (Candido, 2001, Feder & Hofmann 1999, Romanucci et al, 2008, Schlesinger, 1990).

Several studies have investigated the expression levels of HSPs in bees under different stressors (Severson et al, 1990; Alqarni et al, 2019; Zhao et al, 2021; Al-Ghzawi et al, 2022; Abou-Shaara, 2024). Exposure time to heat stress can also affect the expression of HSP subfamily genes, such as Hsp40 in *Apis cerana cerana* (Li et al, 2020). While few amino acid sequences are available for HSPs from different bee taxa, HSP60 and HSP83 are more accessible than HSPs with other molecular weights. Therefore, this study focused on these two types of HSPs. Comparative studies on the sequences, structure, and properties of HSPs between different bee taxa are lacking. However, bioinformatics has the potential to detect variations among organisms (Abou-Shaara, 2019, Abou-Shaara and Elbanoby, 2020, Abou-Shaara and Bayoumi, 2021). This study aims to explore the similarities and differences etween HSP60 and HSP83 of various bee taxa, which could help explain variations in HSP gene expression levels among bee species and subspecies. Furthermore, this study presents avenues for exploring HSPs of bees at the molecular level using bioinformatics tools.

MATERIALS AND METHODS

Heat shock proteins

The heat shock proteins (HSPs) were screened from the GenBank (ncbi.nlm.nih.gov/) for different bee taxa. Finally, 18 taxa were considered in this study, 15 out of them belong to Family Apidae, 2 from Family Halictidae, and one from Megachilidae. The molecular weights of proteins were assessed using molecular weight calculator tool (aatbio.com/tools/calculate-peptide-and-protein-molecular-weight-mw). Then two protein types were considered in the study: HSP60 (Table 1), and HSP83 (Table 2), which are the available HSPs for most bee taxa, including those selected in this study.

Table 1: Heat shock proteins with molecular weight about 60kDa (HSP60) for 18 bee taxa.

Tablo 1: On sekiz arı taksonu için moleküler ağırlığı yaklaşık 60kDa (HSP60) olan ısı şok proteinleri.

Family	Scientific name	GenBank
Apidae	*Apis mellifera*	XP_392899.2
Apidae	*Apis mellifera carnica*	KAG9427898.1
Apidae	*Apis mellifera caucasica*	KAG6796173.1
Apidae	*Apis cerana*	AEY60808.1
Apidae	*Apis cerana cerana*	PBC25875.1
Apidae	*Apis laboriosa*	XP_043798027.1
Apidae	*Apis florea*	XP_003691286.1
Apidae	*Melipona quadrifasciata*	KOX67167.1
Apidae	*Bombus affinis*	XP_050588307.1
Apidae	*Bombus huntii*	XP_050485730.1
Apidae	*Bombus impatiens*	XP_003491864.1
Apidae	*Bombus pyrosoma*	XP_043595839.1
Apidae	*Bombus bifarius*	XP_033301540.1
Apidae	*Bombus terrestris*	XP_003399629.2
Apidae	*Bombus vosnesenskii*	XP_033365710.1
Halictidae	*Megalopta genalis*	XP_033338920.1
Halictidae	*Nomia melanderi*	XP_031827026.1
Megachilidae	*Osmia lignaria*	XP_034186903.1

Table 2: Heat shock proteins with molecular weight about 83kDa (HSP83) for 18 bee taxa.
Tablo 2: On sekiz arı taksonu için moleküler ağırlığı yaklaşık 83kDa (HSP83) olan ısı şok proteinleri.

Family	Scientific name	GenBank
Apidae	*Apis mellifera*	NP_001153536.1
Apidae	*Apis mellifera carnica*	KAG9434369.1
Apidae	*Apis mellifera caucasica*	KAG6802498.1
Apidae	*Apis cerana*	AEY61732.1
Apidae	*Apis cerana cerana*	PBC32016.1
Apidae	*Apis laboriosa*	XP_043799257.1
Apidae	*Apis florea*	XP_003694932.1
Apidae	*Melipona quadrifasciata*	KOX70250.1
Apidae	*Bombus affinis*	XP_050583884.1
Apidae	*Bombus huntii*	XP_050481870.1
Apidae	*Bombus impatiens*	XP_003492149.1
Apidae	*Bombus pyrosoma*	XP_043586281.1
Apidae	*Bombus bifarius*	XP_033311555.1
Apidae	*Bombus terrestris*	XP_003396897.1
Apidae	*Bombus vosnesenskii*	XP_033352687.1
Halictidae	*Megalopta genalis*	XP_033328903.1
Halictidae	*Nomia melanderi*	XP_031847184.1
Megachilidae	*Osmia lignaria*	XP_034173570.1

Analysis of amino acids and nucleotides.

The amino acid compositions of proteins were assessed using the molecular weight calculator (aatbio.com/tools/calculate-peptide-and-protein-molecular-weight-mw). The amino acid sequences were translated into approximate nucleotide sequences using an online translation tool (bioinformatics.org/sms2/rev_trans.html). Then, the number of each nucleotide type and %G~C were calculated using sequence analysis tool (sciencebuddies.org/science-fair-

projects/references/genomics-g-c-content-calculator). Tables comparing between proteins of the studied bees were used to summarize the results.

Conserved domains and motifs

The conserved domains in the sequences were identified using tool “identify conserved domains” from ncbi.nlm.nih.gov/Structure. Also, motifs were screened in the studied proteins using MOTIF Search (genome.jp/tools/motif/).

Protein properties

Protein analysis options available from bioinformatics.org were utilized to figure out variations among proteins of the studied bees. This contained two parameters: (1) The GRAVY value or grand average of hydropathy for the protein sequences (bioinformatics.org/sms2/protein_gravy.html). The GRAVY value was calculated according to Kyte and Doolittle (1982), and (2) Protein Isoelectric Point (bioinformatics.org/sms2/protein_iep.html), which calculates the theoretical isoelectric point for the protein sequences. The protein isoelectric point can help in specifying the approximately location of a particular protein on a 2-D gel.

The 3D structure of the protein

The website https://www.rcsb.org was used to find out the most identical 3D structure of proteins used in the study. The computed structure models were included in the search criteria. Then, the similarities between the studied bees were spotted.

RESULTS

Analysis of amino acids and nucleotides

The amino acid compositions of different bee taxa for HSP60 were mostly identical, except for some cases where variations were observed (Fig. 1). The identical compositions were found in *A. mellifera*, *A. m. carnica*, *A. m. caucasica*, *A. cerana*, *A. laboriosa*, and *A. florea*, as well as in *B. affinis*, *B. huntii*, *B. impatiens*, *B. bifarius*, *B. terrestris*, and *B. vosnesenskii*. It is noteworthy that some species belonging to the genus *Apis* and *Bombus* are 100% identical to each other. The observed variations (Fig. 2) were only in Alanine and Valine (*M. genalis*), Arginine (*A. c. cerana*), Aspartic acid, Glutamic acid, Leucine (*M. quadrifasciata*), Cysteine (*O. lignaria*), Isoleucine, Phenylalanine (*B. pyrosoma*), and Threonine (*N. melanderi*). Additionally, clear variations were observed in Serine.

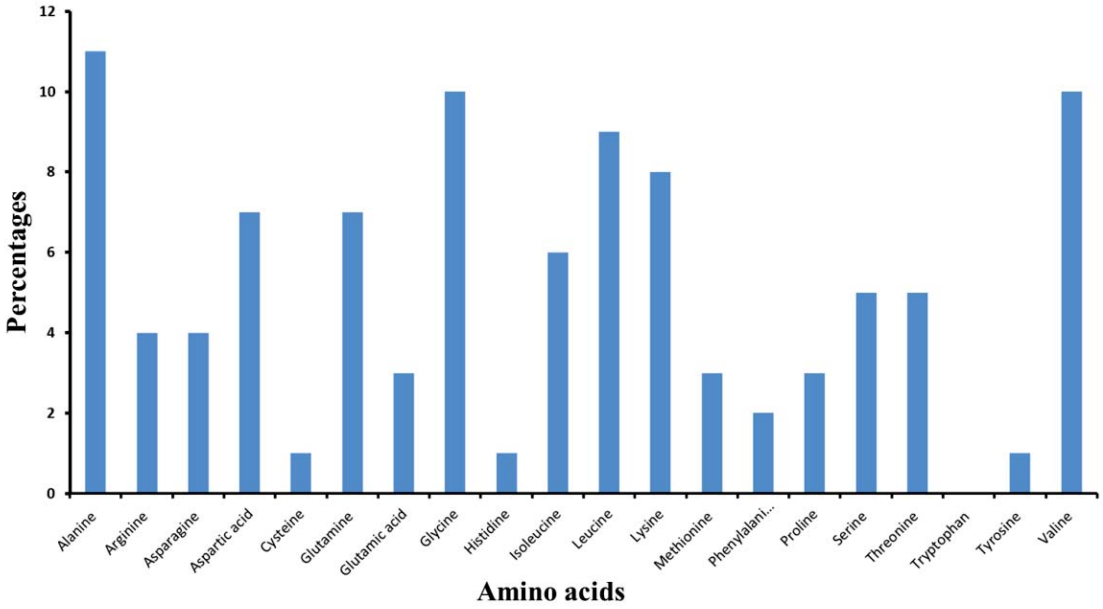

Fig. 1: Percentages of amino acids in the heat shock proteins (HSP60) of the studied bee taxa, except those with unique values for some amino acids listed in Fig.2

Şekil 1: Şekil 2'de listelenen bazı amino asitler için benzersiz değerlere sahip olanlar hariç, çalışılan arı taksonlarının ısı şok proteinlerindeki (HSP60) amino asit yüzdeleri

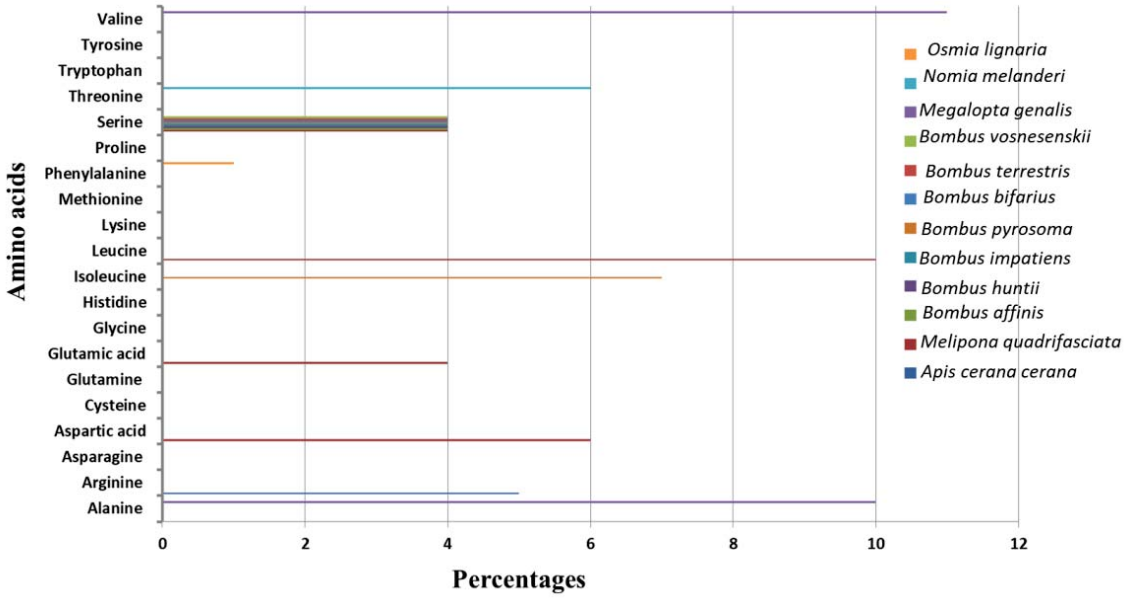

Fig. 2: Unique percentages of amino acids in the heat shock proteins (HSP60) of the studied bee taxa

Şekil 2: Çalışılan arı taksonlarının ısı şok proteinlerindeki (HSP60) amino asitlerin benzersiz yüzdeleri

The amino acid compositions of different bee taxa for HSP83 showed mostly identical compositions between *A. mellifera*, *A. m.* carnica, and *A. cerana cerana*, between *A. cerana* and *A. laboriosa*, between *B. huntii*, *B.* impatiens, and *B. bifarius*, and between *B. pyrosoma*, *B.* terrestris, and *B. vosnesenskii* (Fig. 3). However, some variations were observed in one or more amino acids among the remaining taxa with only 1% difference among them (Fig. 4).

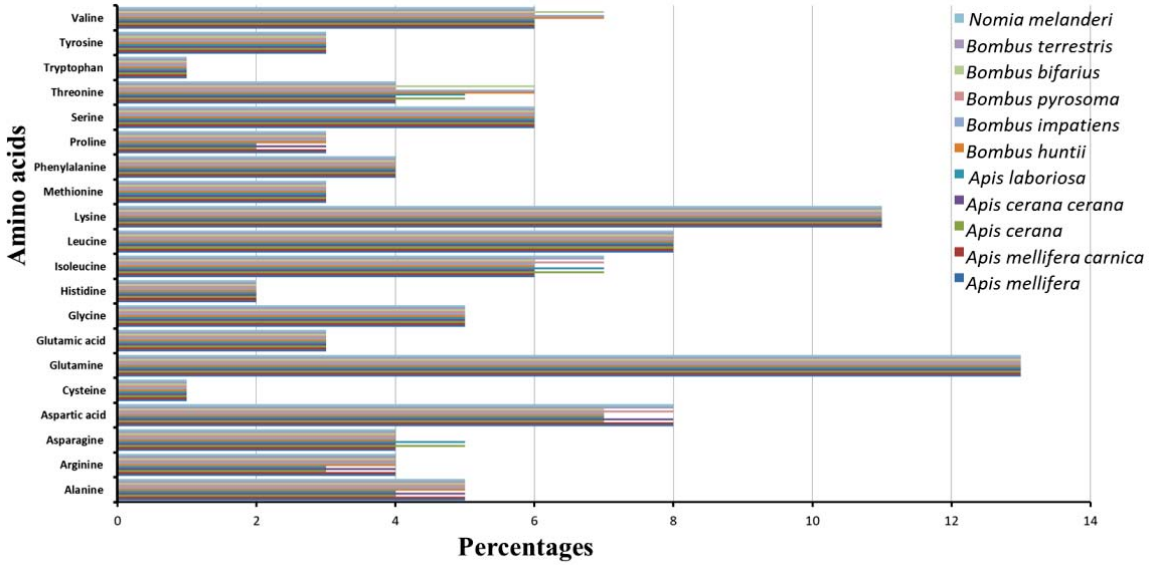

Fig. 3: Percentages of amino acids in the heat shock proteins (HSP83) of the studied bee taxa with identical percentages

Şekil 3: Çalışılan arı taksonlarının ısı şok proteinlerindeki (HSP83) amino asit yüzdeleri ve aynı yüzdeler

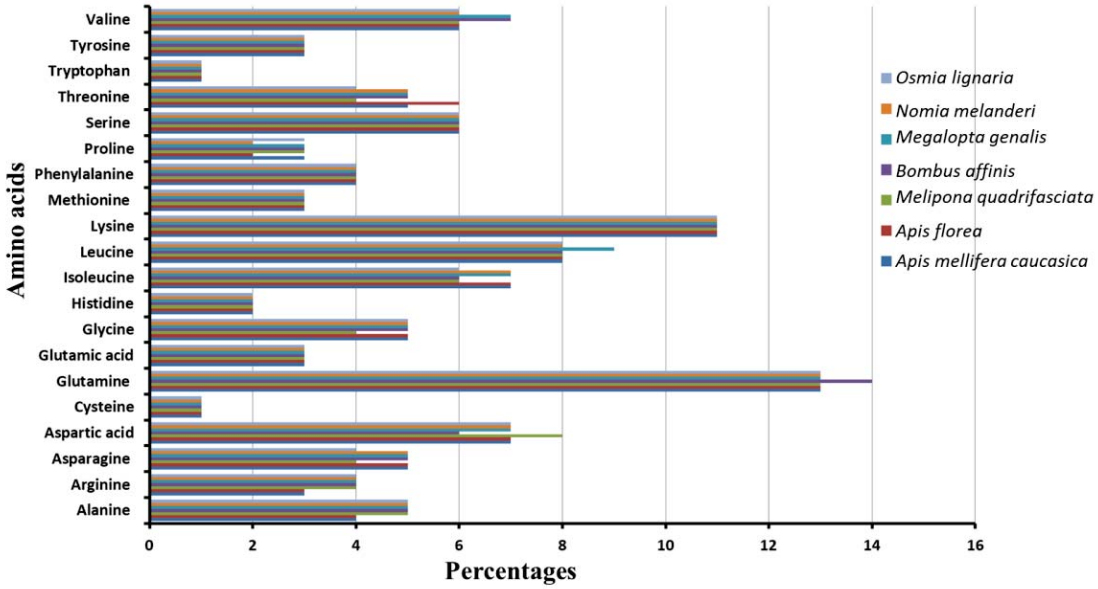

Fig. 4: Bee taxa with variations in their percentages of amino acids in the heat shock proteins (HSP83)

Şekil 4: Isı şok proteinlerindeki (HSP83) amino asit yüzdelerinde farklılık gösteren arı taksonları

The sequence analysis of HSP60 is presented in Table 3, revealing high G content (587-602), followed by A (438-444), then C (389-404), and finally T (282-291) for all bee taxa. The %G~C value ranged from 57.4 to 58%. Only *A. mellifera*, *A. m. carnica*, *A. m. caucasica*, and A. cerana had identical values, while the other taxa showed variations in their sequences.

The sequence analysis of HSP83 is presented in Table 4. All bee taxa had high A content (730-759), followed by G (572-592), then C (406-419), and finally T (415-429). The %G~C value ranged from 44.9-46.7%. The identical values were only to *B. pyrosoma*, *B. terrestris*, and *B. vosnesenskii*, and between *A.m. carnica*, and *A.cerana* cerana. The other taxa showed variations in their sequences.

Table 3: Sequence analysis of the heat shock protein (HSP60) of different bee taxa

Tablo 3: Farklı arı taksonlarının ısı şok proteininin (HSP60) sekans analizi

Species	A	T	G	C	%G~C
Apis mellifera	440	288	589	393	57.4
Apis mellifera carnica	440	288	589	393	57.4
Apis mellifera caucasica	440	288	589	393	57.4
Apis cerana	440	288	589	393	57.4
Apis cerana cerana	444	291	590	400	57.4
Apis laboriosa	439	287	591	393	57.5
Apis florea	439	288	591	392	57.5
Melipona quadrifasciata	442	286	602	404	58
Bombus affinis	439	287	592	392	57.5
Bombus huntii	443	288	596	392	57.5
Bombus impatiens	440	287	590	393	57.5
Bombus pyrosoma	444	288	587	391	57.2
Bombus bifarius	442	288	591	389	57.3
Bombus terrestris	439	287	592	392	57.5
Bombus vosnesenskii	442	287	591	390	57.4
Megalopta genalis	438	286	593	390	57.6
Nomia melanderi	443	282	591	391	57.5
Osmia lignaria	443	284	593	390	57.5

Table 4: Sequence analysis of the heat shock protein (HSP83) of different bee taxa

Tablo 4: Farklı arı taksonlarının ısı şok proteininin (HSP83) sekans analizi

Species	A	T	G	C	%G~C
A. mellifera	742	428	592	410	46.1
A. mellifera carnica	744	428	591	409	46
A. mellifera caucasica	757	427	564	406	45
A. cerana	758	428	558	407	44.9
A. cerana cerana	744	428	591	409	46
A. laboriosa	759	427	558	407	44.9
A. florea	756	427	559	409	45
M. quadrifasciata	736	418	572	392	45.5
B. affinis	746	415	581	412	46.1
B. huntii	739	418	581	416	46.3
B. impatiens	738	417	580	416	46.3
B. pyrosoma	746	429	592	408	46
B. bifarius	739	418	580	417	46.3
B. terrestris	746	429	592	408	46
B. vosnesenskii	746	429	592	408	46
M. genalis	730	418	588	418	46.7
N. melanderi	752	415	583	419	46.2
O. lignaria	732	417	592	413	46.7

Table 5: The motifs in the sequences of the heat shock proteins (HSP60 and HSP83) of different bee taxa

Tablo 5: Farklı arı taksonlarının ısı şok proteinlerinin (HSP60 ve HSP83) dizilerindeki motifler

	HSP60		**HSP83**	
Species	Interval	Interval 1	Interval 2	Interval 3
A. mellifera	42to540	192to706	36to189	35to155
A. mellifera carnica	42to540	192to706	36to189	35to155
A. mellifera caucasica	42to540	191to700	35to188	34to154
A. cerana	42to540	192to699	35to188	34to154
A. cerana cerana	42to540	192to706	36to189	35to155
A. laboriosa	42to540	191to699	34to154	34to154
A. florea	42to540	191to699	35to188	34to154
M. quadrifasciata	270to549	172to688	36to151	35to157
B. affinis	42to540	192to702	36to189	35to155
B. huntii	42to540	192to701	36to189	35to155
B. impatiens	42to540	191to700	35to188	34to154
B. pyrosoma	42to540	192to707	36to189	35to155
B. bifarius	42to540	192to701	36to189	35to155
B. terrestris	42to540	192to707	36to189	35to155
B. vosnesenskii	42to540	192to707	36to189	35to155
M. genalis	42to540	191to701	35to188	34to154
N. melanderi	42to540	191to714	35to188	34to154
O. lignaria	42to540	191to700	35to188	34to154

Conserved domains and motifs

The conserved domains in the sequences of HSP60 showed high identical values with intervals ranging from 23 to 541, except for *M. quadrifasciata* with an interval from 69 to 550. For HSP83, the conserved domains in the sequences were identical in some bee taxa with intervals ranging from 13 to 717, 13 to 718, 13 to 723, 14 to 724, and 14 to 725. However, *M. quadrifasciata* had more distinct conserved domain intervals than the other bee taxa, with an interval from 14 to 706. The variations observed in the conserved domains were higher in HSP83 than in HSP60.

The motifs present in the sequences of heat shock proteins (HSP60 and HSP83) of different bee taxa are listed in Table 5. The motifs of HSP60 belonged

to one protein family (Pfam: Cpn60_TCP1) for all taxa except *M. genalis* and *N.* melanderi, which belonged to two protein families (Pfam: Cpn60_TCP1 and AcnX_swivel_put). The interval for motifs (Pfam: Cpn60_TCP1 range from 42 to 540, AcnX_swivel_put range from 233 to 283; E-value: 1.9e-41 to 7.6e-83) in bee taxa for HSP60. *M. genalis* and *N. melanderi* had two motifs compared to other taxa. For HSP83, the motifs belonged mainly to three major protein families (Pfam: HSP90, HATPase_c, and HATPase_c_3). Pfam: HSP90 ranged from 191 to 707 (E-value: 1.2e-230 to 8.5e-231), Pfam: HATPase_c ranged from 35 to 189 (E-value: 1.2e to 14-8e-11), and Pfam: HATPase_c_3 ranged from 34 to157 (E-value: 1.6e-10 to 9e-10).

Protein properties

The GRAVY values for HSP60 ranged from -0.063 to -0.102, with some taxa having identical values. The isoelectric point for HSP60 ranged from 5.26 to 5.89, with identical values for all taxa except *A.cerana cerana*, *M. genalis*, and *O. lignaria* (Table 6). Regarding HSP83, the GRAVY values ranged from -0.661 to -0.714, and variations between taxa were observed. The isoelectric point for HSP83 ranged from 4.61 to 4.73, with few identical values among taxa.

Table 6: The GRAVY and isoelectric point values of the heat shock proteins (HSP60 and HSP83) of different bee taxa

Tablo 6: Farklı arı taksonlarının ısı şok proteinlerinin (HSP60 ve HSP83) GRAVY ve izoelektrik nokta değerleri

	HSP60		HSP83	
Species	GRAVY	Isoelectric Point	GRAVY	Isoelectric Point
A. mellifera	-0.083	5.51	-0.693	4.70
A. mellifera carnica	-0.083	5.51	-0.697	4.68
A. mellifera caucasica	-0.083	5.51	-0.691	4.69
A. cerana	-0.083	5.51	-0.681	4.72
A. cerana cerana	-0.102	5.89	-0.697	4.68
A. laboriosa	-0.084	5.51	-0.697	4.71
A. florea	-0.079	5.51	-0.681	4.70
M. quadrifasciata	-0.080	5.51	-0.714	4.61
B. affinis	-0.072	5.51	-0.675	4.71
B. huntii	-0.068	5.51	-0.662	4.73
B. impatiens	-0.076	5.51	-0.666	4.73
B. pyrosoma	-0.075	5.51	-0.694	4.69
B. bifarius	-0.063	5.51	-0.662	4.73
B. terrestris	-0.072	5.51	-0.694	4.69
B. vosnesenskii	-0.071	5.51	-0.694	4.69
M. genalis	-0.072	5.26	-0.623	4.72
N. melanderi	-0.090	5.51	-0.695	4.71
O. lignaria	-0.084	5.38	-0.661	4.69

The 3D structure of the protein

The results indicate that the 3-D structure of the studied proteins is highly conserved across different bee taxa. Specifically, the structure of HSP60 closely resembles that of *Drosophila* melanogaster, while HSP83 shows high similarity to the structure found in *Bombyx mori* (Fig. 2).

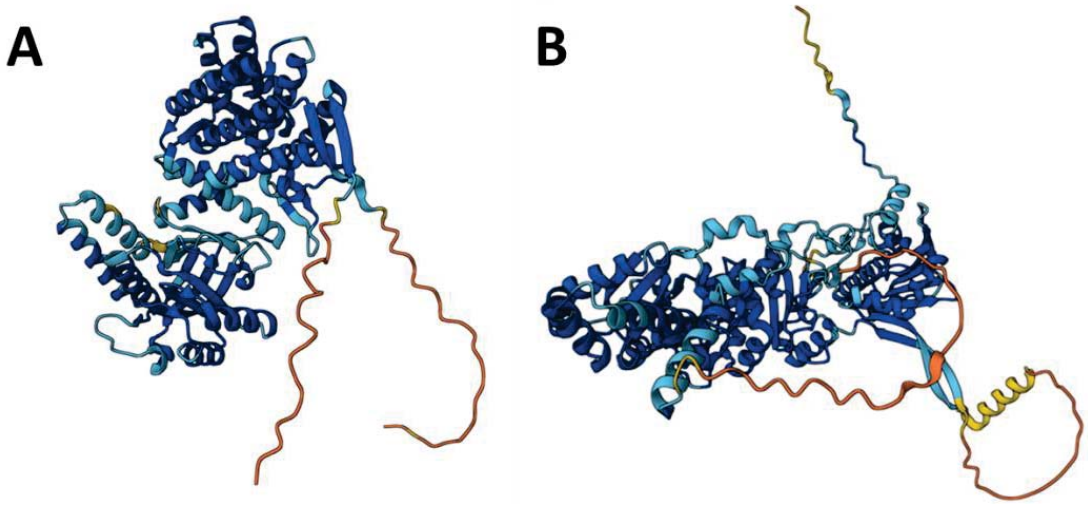

Fig. 5: The most identical 3-D structure of HSP60 (A) (from *Drosophila melanogaster*) and HSP83 (B)(from *Bombyx mori*) of studied bees based on the computed structure models

Şekil 5: Hesaplanan yapı modellerine göre incelenen arıların HSP60 (A) (Drosophila melanogaster'den) ve HSP83 (B) (Bombyx mori'den) en benzer 3 boyutlu yapısı

DISCUSSION

The molecular weight of HSP60 from different bee taxa ranged from 60.27 to 61.44 kDa, which is not precisely 60 kDa. It is possible that the tissue type at which the HSPs were expressed had an impact on their molecular weight since HSP expression can be influenced by the type of tissue (Elekonich, 2009; Abou-Shaara, 2024). Al-Ghzawi et al. (2022) found that HSP60 expression dynamics were more stable in the head than in the thorax after heat stress exposure. In this study, the HSPs used were expressed in the whole body or specific body parts, such as the abdomen or thorax, and not from the same tissue for all bee taxa. HSP60 has a role in mitochondrial protein homeostasis, cellular proliferation, and apoptosis (Bukau and Horwich, 1998, Caruso Bavisotto et al., 2020, Henderson et al., 2013, Meng et al., 2018). As a mitochondrial chaperone, HSP60 occurs in the mitochondria and other parts of eukaryotic cells (Gupta, 1995, Parnas et al., 2012). The functions of HSP60 are expected to be similar in all bee taxa, which could explain the few variations in amino acid compositions, with some species/subspecies having highly identical values. The amino acid compositions had the same pattern based on the percentage of each amino acid, with alanine, glycine, and valine being the highest in percentage for all bee taxa. The amino acid compositions contained essential amino acids, except for tryptophan. The sequences showed a similar distribution pattern for G, A, C, and T, with somewhat similar %G~C. The conserved domains in the sequences showed high identical values for HSP60, except for *M. quadrifasciata*. The results showed the presence of only one motif from Pfam: Cpn60_TCP1, except for *M. genalis* and *N. melanderi*, which had two motifs from Pfam: Cpn60_TCP1 and AcnX_swivel_put. Such low variations between HSP60 from different bee taxa could be explained by the high similarity in the amino acid compositions.

In a similar way to HSP60, the exact molecular weight of HSP83 ranged from 81.63 to 83.64 KDa. HSP83 as a molecular chaperone protein belongs to HSP90 family (Arya et al., 2007, Li et al., 2007, Rutherford and Lindquist 1998) has role in protein chaperoning (Ray et al., 2019). High identical amino acid compositions were detected in some cases. All the 9 essential amino acids were detected in the amino acid compositions of all bee taxa. The highest amino acids in their percentages were glutamine, lysine, and Leucine. The sequences showed similar distribution pattern for nucleotides, with somewhat similar %G~C. However, the identical values were only between few taxa. Additionally, the conserved domains in the sequences were identical in some bee taxa for HSP83. The motifs belonged to three protein families (Pfam: HSP90, HATPase_c, and HATPase_c_3). In general, HSP83 showed high

variations among bee taxa. This can be explained by the complex roles of this protein on the cell level.

The variations in HSP83 are greater than those observed in HSP60, indicating that increasing the molecular weight of a protein can lead to higher variability among HSPs in different taxa. The differences in amino acid composition between any two bee taxa were only 1 to 3 amino acids. Both HSP60 and HSP83 contain essential amino acids that are not synthesized by bees and are therefore dietarily essential. However, HSP83 contains 9 essential amino acids, while HSP60 contains only 8 without tryptophan. These variations can be explained by the differences in their functions, with HSP83 expected to have more functions than HSP60. Furthermore, HSP60 has a high content of G, followed by A, C, and T, while HSP83 has a high A content, followed by G, C, and T. The %G~C values were higher in HSP60 than in HSP83. These variations can be explained by the differences in the amino acid composition of the two protein types. The sequences of HSP83 show greater variation than those of HSP60. All bee taxa showed variations in the sequences of Hps83 with less identical values compared to HSP60. HSPs are known to have highly conserved sequences and domains among divergent organisms (Schlesinger, 1990). So, the sharing of identical values between some bee taxa in this study can be attributed to this conservation.

The conserved domains showed high variations in HSP83 than HSP60. A motif, critical for domain function, is a short conserved sequence pattern and made up of connectivity between secondary structural pieces of protein. A motif is linked with specific protein structural site performing a certain function. The motif was overlapped with the high conserved domains in case of HSP60. No overlap was observed in case of HSP83. The variations between the two protein types in their amino acid composition and sequences can explain the detected conserved domains and motifs.

The negative GRAVY values indicate that HSP60 and HSP83 are hydrophilic, which is consistent with previous studies (Yano et al., 1994; Shamrock et al., 2009). HSP60 has lower GRAVY values than HSP83 but with a higher isoelectric point, which suggests that HSP83 is more hydrophilic than HSP60. The difference in isoelectric points also indicates that HSP60 and HSP83 have different locations on a 2-D gel, which also can be linked to the variations in their molecular weights. HSP83 showed more variations among bee taxa than HSP60 across all measured parameters. The few variations in HSP60 may be related to its chaperone activity in preventing protein misfolding under stressful conditions (Brocchieri and Karlin 2000, Cappello et al., 2014), while HSP83 has more complex roles (Abou-Shaara 2024) such as juvenile hormone signaling (He et al., 2014) and ovary development (Chen et al, 2018). Therefore, the observed variations in HSP83 compared to HSP60 are likely due to their different functions. The 3-D structures of proteins are determined by their amino acid sequences and bond types, and the maintenance of this structure is essential for their functionality. The study found highly conserved 3-D structures of HSPs in the studied bee taxa, with each protein having a unique shape.

Conclusion

Heat shock proteins (HSPs) are widely studied in different bee taxa, and their expression levels vary among individuals from the same or different taxa. This study aimed to investigate the sequence and amino acid level properties of two HSP types (60 and 83) in 18 bee taxa. The study found highly conserved 3-D structures and amino acid compositions in both HSPs, with some species/subspecies having highly identical values, especially for bees from genus *Apis* and *Bombus*. However, HSP83 showed greater variations among bee taxa than HSP60, and the conserved domains showed high variations in HSP83 than HSP60. The study suggests that the observed variations in HSPs could explain the potential variations in their response levels among bee taxa under stress conditions. Therefore, it is important to not only study the expression of HSPs but also to analyze their sequences and perform more protein-level analyses. The study also highlights the impact of the rearing environment of bees on the properties of HSPs, which requires further investigation. Future comparative studies among bee taxa under the same experimental conditions are highly recommended. The analysis of phylogenetic relationships could be helpful when comparing HSPs expressed in the same tissues and under the same stressors for various taxa, which need further studies. This study encourages researchers to conduct more investigations on HSPs, especially under current climate change challenges.

Author contribution: The author designed, performed, analyzed the data, wrote and revised the manuscript.

Conflict of Interest: No conflict of interests to be reported.

Ethical issue: Not applicable because this study on honey bees and not animals or humans.

Source of Finance: Not applicable.

REFERENCES

Abou-Shaara H.F. Expectations about the potential impacts of climate change on honey bee colonies in Egypt. J. Apic., 2016; 31, 157-164.

Abou-Shaara H.F. Utilizing bioinformatics to detect genetic similarities between African honey bee subspecies. J. Genet., 2019; 98, 96. doi:10.1007/s12041-019-1145-7.

Abou-Shaara H.F. The response of heat shock proteins in honey bees to abiotic and biotic stressors. J. Therm. Biol., 2024; 119, 103784.

Abou-Shaara H.F., & Bayoumi S.R. Genetic variations and relationships between deformed wing virus strains infesting honey bees based on structural proteins. Biologia, 2021; 76(12), 3865-3873.

Abou-Shaara H.F., & Elbanoby M.I.A bioinformatics study to detect the genetic characteristics of *Vespa* hornets (Hymenoptera: Vespidae). J. Entomol. Res. Soc., 2020; 22, 227-237.

Abou-Shaara H.F., Al-Ghamdi A.A. & Mohamed, A.A. Tolerance of two honey bee races to various temperature and relative humidity gradients. Env. Exp. Biol., 2012; 10, 133-138.

Abou-Shaara H.F., Al-Khalaf A.A. Using Maximum Entropy Algorithm to Analyze Current and Future Distribution of the Asian hornet, *Vespa velutina*, in Europe and North Africa Under Climate Change Conditions. J. Entomol. Res. Soc., 2022; 24, 07-21.

Abou-Shaara H.F., Amiri E. & Parys K.A. Tracking the effects of climate change on the distribution of Plecia nearctica (Diptera, Bibionidae) in the USA using MaxEnt and GIS. Diversity 2022; 14, 690.

Abou-Shaara H.F., Owayss A.A., Ibrahim Y.Y. & Basuny N.K. A review of impacts of temperature and relative humidity on various activities of honey bees. Insect. Soc., 2017; 64, 455-463.

Al-Ghzawi A.A.M.A., Al-Zghoul M.B., Zaitoun S., Al-Omary I.M. & Alahmad N.A. Dynamics of heat shock proteins and heat shock factor expression during heat stress in daughter workers in pre-heat-treated (rapid heat hardening) *Apis mellifera* mother queens. J. Ther. Biol., 2022; 104, 103194.

Alqarni A.S. Tolerance of summer temperature in imported and indigenous honeybee *Apis mellifera* L. races in central Saudi Arabia. Saudi J. Biol. Sci., 2022; 13, 123–127.

Alqarni A.S., Ali H., Iqbal J., Owayss A.A. & Smith B.H. Expression of heat shock proteins in adult honey bee (*Apis mellifera* L.) workers under hot-arid subtropical ecosystems. Saudi J. Biol. Sci., 2019; 26, 1372–1376.

Arretz P.V. & Macfarlane R.P. The introduction of Bombus ruderatus to Chile for red clover pollination. Bee World, 1986; 67, 15-22.

Arya R., Mallik M. & Lakhotia S.C. Heat shock genes - integrating cell survival and death. J. Biosci., 2007; 32, 595–610.

Aslan C.E., Liang C.T., Galindo B., Kimberly H. & Topete W. The role of honey bees as pollinators in natural areas. Nat. Areas J. 2016; 36, 478-488.

Bellard C., Bertelsmeier C., Leadley P., Thuiller W. & Courchamp F. Impacts of climate change on the future of biodiversity. Ecol. Lett. 2012; 15, 365–377.

Bernauer O.M., Tierney S.M. & Cook J.M. Efficiency and effectiveness of native bees and honey bees as pollinators of apples in New South Wales orchards. Agri. Ecosyst. Environ. 2022; 337, 108063.

Blažytė-Čereškienė L., Vaitkevičienė G., Venskutonytė S. & Būda, V. Honey bee foraging in spring oilseed rape crops under high ambient temperature conditions. Žemdirb. (Agric.) 2010¸97, 61-70.

Brocchieri L. & Karlin S. Conservation among HSP60 sequences in relation to structure, function, and evolution. Prot. Sci. 2000; 9, 476-486.

Bukau B. & Horwich A.L. The Hsp70 and Hsp60 chaperone machines. Cell 1998; 92, 351-366.

Candido E.P.M. Heat shock proteins. In: Brenner, S., Miller, J.H. (Eds.), Encyclopedia of Genetics. Academic Press, New York, 2001¸914–915.

Cappello F., Marino Gammazza, A., Palumbo Piccionello A., Campanella C., Pace A., Conway de Macario, E. & Macario, A.J. Hsp60 chaperonopathies and chaperonotherapy: targets and agents. Exp. Opin. Ther. Tar. 2014¸18, 185-208.

Caruso Bavisotto C., Alberti G., Vitale A.M., Paladino L., Campanella C., Rappa, F., Gorska M., Conway de Macario E., Cappello F., Macario A.J.L. & Marino Gammazza A.. Hsp60 post-translational modifications: functional and pathological consequences. Front. Mol. Biosci. 2020¸7, 95.

Chen D., Wang S., Tao X., Zhou L., Wang J., Sun F., Sun M., & Gao X. Hsp83 regulates the fate of germline stem cells in Drosophila ovary. J. Genet. Genom. 2018¸45, 219-222.

Csermely P., Schnaider T., So C., Prohászka Z. & Nardai G. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol. Therapeut. 1998¸79, 129-168.

David J.R., Araripe L.O., Chakir M., Legout H., Lemos B., Petavy G., Rohmer C., Joly D., Moreteau B. Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding Drosophila climatic adaptations. J. Evol. Biol. 2005¸18, 838–846.

Du Y., Ma A., Zha Q.H., Ma G. & Yang H.P. Effects of heat stress on physiological and biochemical mechanisms of insects: a literature review. Acta Ecol. Sin. 2007¸27, 1565–1572.

Easterling D.R., Meehl G.A., Parmesan C., Changnon S.A., Karl, T.R. & Mearns, L.O. Climate extremes: observations, modeling, and impacts. Sci. 2000; 289, 2068–2074.

Elekonich M.M. Extreme thermotolerance and behavioral induction of 70-kDa heat shock proteins and their encoding genes in honey bees. Cell Stress Chaperones 2009; 14, 219-226.

Engel M.S. The taxonomy of recent and fossil honey bees (Hymenoptera, Apidae, Apis). J. Hymenoptera Res. 1999¸ 8, 165–196.

Feder M.E. & Hofmann G.E. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 1999; 61, 243–282.

Gibney E., Gault J. & Williams J. The use of stress proteins as a biomarker of sublethal toxicity: induction of heat shock protein 70 by 2-isobutyl piperidine and transition metals at sub-lethal concentrations. Biomarkers 2001¸6, 204-217.

Gupta R.S. Evolution of the chaperonin families (Hsp60, Hsp 10 and Tcp-1) of proteins and the origin of eukaryotic cells. Mol. Microbiol. 1995; 15, 1–11.

Hausmann S.L., Petermann J.S. & Rolff J. Wild bees as pollinators of city trees. Insect Conserv. Div. 2016; 9, 97-107.

He Q., Wen D., Jia Q., Cui C., Wang J., Palli S.R., Li S. Heat shock protein 83 (Hsp83) facilitates methoprene-tolerant (Met) nuclear import to modulate juvenile hormone signaling. J. Biol. Chem. 2014; 289, 27874-27885.

Heard T.A. The role of stingless bees in crop pollination. Ann. Rev. Entomol. 1999; 44, 183-206.

Henderson B., Fares M.A. & Lund P.A. Chaperonin 60: a paradoxical, evolutionarily conserved protein family with multiple moonlighting functions. Biol. Rev. Camb. Phil. Soc. 2013; 88, 955–987.

Hoffmann A.A., Sørensen J.G. & Loeschcke V. Adaptation of Drosophila to temperature extremes: bringing together quantitative

and molecular approaches. J. Therm. Biol. 2003; 28, 175–216.

Hung K.L.J., Kingston J.M., Albrecht M., Holway D.A. & Kohn J.R. The worldwide importance of honey bees as pollinators in natural habitats. Proc. Royal Soc. B: Biol. Sci. 2018; 285, 20172140.

Ilyasov R.A., Lee M.L., Takahashi J.I., Kwon H.W. & Nikolenko A.G. A revision of subspecies structure of western honey bee Apis mellifera. Saudi J. Biol. Sci. 2020; 27, 3615-3621.

Ilyasov R.A., Lee M.-L., Yunusbaev U.B., Nikolenko A.G. & Kwon H.-W. Estimation of C-derived introgression into A. m. mellifera colonies in the Russian Urals using microsatellite genotyping. Genes and Genom. 2020; 42 (9), 987–996.

Karl I., Stoks R., De Block, M., Janowitz S. & Fischer K. Temperature extremes and butterfly fitness: conflicting evidence from life history and immune function. Global Change Biol. 2011; 17, 676–687.

Kearns C.A. & Inouye D.W. Pollinators, flowering plants, and conservation biology. Bioscience 1997; 47, 297-307.

Kevan P.G. & Viana B.F. The global decline of pollination services. Biodiversity, 2003; 4, 3-8.

Koo J., Son T.G., Kim S.Y. & Lee K.Y. Differential responses of *Apis mellifera* heat shock protein genes to heat shock, flower-thinning formulations, and imidacloprid. J. Asia Pac. Entomol. 2015; 18, 583–589.

Kyte J. & Doolittle R.F. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 1982; 157, 105-132.

Lautenbach S., Seppelt R., Liebscher J. & Dormann, C.F. Spatial and temporal trends of global pollination benefit. PLoS One 2012; 7, e35954.

Le Conte, Y. & Navajas M. Climate change: impact on honey bee populations and diseases. Revue Sci. Tec. 2008; 27, 499-510.

Li G., Zhao, H. Guo, H., Wang Y., Cui, X., Li H., Guo X. Analyses of the function of DnaJ family proteins reveal an underlying regulatory mechanism of heat tolerance in honeybee. Sci. Total Env. 2020; 716, 137036.

Martinet B., Lecocq, T., Smet, J. & Rasmont, P. A protocol to assess insect resistance to heat waves, applied to bumblebees (*Bombus Latreille*, 1802). PloS One 2015; 10, e0118591.

Meng Q., Li B.X. & Xiao X. Toward developing chemical modulators of Hsp60 as potential therapeutics. Front. Mol. Biosci. 2018; 5, 35.

Mosser D.D., Caron A.W., Bourget L., Denis-Larose C. & Massie B. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol. Cell Biol. 1997; 17, 5317–5327.

Nazir A., Saxena D.K. & Kar Chowdhuri D. Induction of hsp70 in transgenic Drosophila: biomarker of exposure against phthalimide group of chemicals. Biochim. Biophys. Acta 2003; 1621, 218–225.

Nicholls C.I. & Altieri M.A. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agron. Sust. Develop. 2013; 33, 257-274.

Oyen K.J., Giri S. & Dillon M.E. Altitudinal variation in bumble bee (Bombus) critical thermal limits. J. Thermal Biol. 2016; 59, 52-57.

Parnas A., Nisemblat S., Weiss C., Levy-Rimler G., Pri-Or A., Zor T., Lund P.A., Bross P., Azem A. Identification of elements that dictate the specificity of mitochondrial Hsp60 for its co-chaperonin. PLoS One 2012; 7, e50318.

Pettis J.S., Rice N., Joselow K., vanEngelsdorp, D. & Chaimanee V. Colony failure linked to low sperm viability in honey bee (*Apis mellifera*) queens and an exploration of potential causative factors. PLoS One 2016; 11, e0147220.

Qi, Y., Wang H., Zou, Y., Liu C., Liu Y., Wang Y. & Zhang W. Over-expression of mitochondrial heat shock protein 70 suppresses programmed cell death in rice. FEBS Lett. 2011; 585, 231-239.

Ray M., Acharya S., Shambhavi S. & Lakhotia S.C. Over-expression of Hsp83 in grossly

depleted hsromega lncRNA background causes synthetic lethality and l(2)gl phenocopy in Drosophila. J. Biosci. 2019; 44.

Reddy P.V., Verghese A. & Rajan V.V. Potential impact of climate change on honeybees (*Apis* spp.) and their pollination services. Pest Manag. Horticul. Ecosyst. 2012; 18, 121-127.

Romanucci M., Bastow T. & Della Salda L. Heat shock proteins in animal neoplasms and human tumours–a comparison. Cell Stress Chaperones 2012; 13, 253–262.

Rutherford S.L. & Lindquist S. Hsp90 as a capacitor for morphological evolution. Nature 1998; 396, 336.

Sales K., Vasudeva R., Dickinson M.E., Godwin J.L., Lumley A.J., Michalczyk L., Hebberecht,L., Thomas P., Franco A. & Gage M.J.G. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nat. Commun. 2018; 9, 4771.

Sangle P.M., Satpute S.B., Khan F.S. & Rode N.S. Impact of climate change on insects. Trends Biosci 2015; 8, 3579-3582.

Schlesinger M.J. Heat shock proteins. J. Biol. Chem. 1990; 21, 12111-12114.

Severson D.W., Erickson, E.H., Williamson, J.L., Aiken, J.M. Heat stress induced enhancement of heat shock protein gene activity in the honey bee (*Apis mellifera*). Experientia 1990; 46, 737-739.

Shamrock V.J., Duval J.F., Lindsey G.G., & Gaboriaud F. The role of the heat shock protein Hsp12p in the dynamic response of Saccharomyces cerevisiae to the addition of Congo red. FEMS yeast research, 2009; 9(3), 391-399.

Sheppard W.S. & Meixner M.D. *Apis mellifera pomonella*, a new honeybee subspecies from Central Asia. Apidologie 2003; 34, 367–375.

Stabentheiner A., Kovac H. & Brodschneider R. Honeybee colony thermoregulation–regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. PLoS One 2010; 5, e8967.

Tautz J., Maier S., Groh C., R¨ossler W., Brockmann A. Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proc. Natl. Acad. Sci. 2003; 100, 7343–7347.

Thomann M., Imbert E., Devaux C., Cheptou P.O. Flowering plants under global pollinator decline. Trend. Plant Sci. 2013; 18, 353-359.

Thomas C.D., Cameron A., Green R.E., Bakkenes M., Beaumont L.J., Collingham, Y.C., Erasmus B.F., De Siqueira M.F., Grainger A., Hannah L., Hughes L., Huntley B., Van Jaarsveld A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson A.T., Phillips O.L., Williams S.E. Extinction risk from climate change. Nature 2004; 427, 145–148.

Vermeulen C.J. & Loeschcke V. Longevity and the stress response in Drosophila. Exp. Gerontol. 2007; 42, 153–159.

Whitehorn P.R., Tinsley M.C, Brown M.J., Goulson D. Investigating the impact of deploying commercial Bombus terrestris for crop pollination on pathogen dynamics in wild bumble bees. J. Apic. Res. 2013; 52, 149-157.

Winfree R, Gross, B.J. & Kremen C. Valuing pollination services to agriculture. Ecol. Econ. 2011; 71, 80-88.

Yano K, Hasegawa T, Kuboi R, Komasawa I, & Tsuchido T. Characterization of surface properties of heat shock proteins for the separation using aqueous two-phase systems. J. Chem. Eng. Japan, 1994; 27(6), 808-814.

Zambra E, Martinet B, Brasero N, Michez D. & Rasmont P. Hyperthermic stress resistance of bumblebee males: test case of Belgian species. Apidologie 2020; 51, 911-920.

Zhao H, Li G, Guo D, Li H, Liu Q, Xu B.& Guo X. Response mechanisms to heat stress in bees. Apidologie 2021; 52, 388-399.