STRESS PROTEIN RESPONSES in HONEY BEES: IS IT USEFUL TO MEASURE STRESS RESPONSES OF INDIVIDUAL BEES IN THE HIVE?

Bal Arılarında Stress Protein Tepkileri: Kovandaki Her Bir Arının Strese Tepkilerinin Ölçülmesi Kullanışlı mı?

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Key Words: *Apis mellifera*, chemicals, honey bees, stress proteins

Anahtar Kelimeler: *Apis mellifera,* kimyasallar, bal arısı, stres proteinleri

ABSTRACT

Eusociality provides honey bees a broad repertoire of responses, through a colony's division of labor, to maintain hive homeostasis in the countenance of environmental perturbations. The hive dynamics instrumented by workers must be balanced against losses during periods of stress. Stress proteins, a component of the cellular stress response that is already characterized in species from bacteria to man, provide molecular protection against many stressors at the organismal level of biological organization. A capacious stress protein literature reveals several general patterns. Exposure to sublethal stress increases cellular stress protein concentrations and improves survival to subsequent stress. While promoting survival during periods of stress, over-expression of stress proteins during development may diminish expression of performance traits important later in life under different circumstances. The relatively few studies that have investigated stress responses in bees reveal relationships with abiotic stress (*i.e.* temperature, toxins) and oxidative stress associated with flight and alcohol consumption. Given the economic importance of the honey bee and the need to better understand how agricultural factors (*e.g.*, hive management practices, pesticides, natural enemies) affect colony performance, investigations of the association between the stress response and performance traits in individual bees should be pursued in the future.

INTRODUCTION

The seminal works of Malthus (1798) and Darwin (1859) changed the way we view both nature and the natural history of species. Their model focuses attention on the ecology of how organisms survive, which is largely due to their response to stress induced by elements of the physical and biotic environments (*i.e*., environmental stressors). In essence, organisms seek to maintain an acceptable homeostasis through their response to stressors. Honey bees are a particularly interesting organism with which to study how species maintain homeostasis in the face of environmental stressors because they are eusocial. The hoarding of honey by honey bees is an excellent example of how the colony faces seasonal nectar shortages, and has been exploited by humans for millennia (Crane 1999). However, honey bee response to this stressor is not simply a colony level function.

Surplus originates in part through forager behaviors that involve both energy maximization principles and fierce competition strategies against other nectivore species (reviewed in: Sanderson & Wells 2005). Although we do see foragers selecting the flowers with the greatest net reward, they also partition the work force by flower type and thus collect from all nectar sources in the environment, which leaves little for their competitors (Wells & Wells 1983, 1986; Hill *et al*. 1997; 2001; Sanderson *et al*. 2006). Even in non-native environments, the degree to which honey bees are the source of, or target of, stress remains a subject of debate (Waser *et al*. 1996; Butz Huryn 1997; Goulson 2003).

Hive defense has apparently differentiated among subspecies of *A. mellifera* and aggression is known to vary among subspecies (Abramson & Aquino 2002). For example, Africanized bees are very aggressive and Caucasian bees are known for their gentle behavior (Cakmak & Wells 1996). In addition, coordination of hive defenses varies among subspecies according to wasp predator threat in the endemic regions of the subspecies (Cakmak & Wells 1998, 2001; Ken *et al*. 2005). These complex colony responses involve the formation of a line or mass of defenders in response to an attacking wasp as bees cease exiting the hive (Breed *et al*. 2004). European honey bee (*Apis mellifera*) population decline ("Colony Collapse Disorder" or CCD) and thepotential impact of CCD on agriculture have also raised public awareness about honey bee parasites and pathogens as biotic stressors (Johnson 2007).

Honey bee homeostasis in the face of environmental stressors occurs at the colony level but with individual actions being important precursors for the colony action to be effective. Cellular and molecular responses to stressors initiate these individual and colony level homeostatic actions. Artificial selection in honey bees shows that quantitative genetic loci control pollen-hoarding and associated traits in honey bees (reviewed in: Page *et al*. 2006. Parasites and pathogens suppress honey bee immune responses (Moret & Schmid-Hempel 2000; Yang & Cox-Foster 2005; 2007), which points to a cellular link. When considering starvation stress, chemical signals among and within hive members alter metabolism and the division of labor to provide starvation resistance to nutritional stress (Fischer & Groziner 2008; Ament *et al*. 2008). Alternative nutrient use among hive members during nutrient stress preserves learning and memory performance among workers for hive function (Matilla & Smith 2008).

Our intent in this article is to review mechanisms of stress protein responses and how the stress protein response may provide stress tolerance in honey bees at the organismal and cellular-molecular levels of organization while serving as a useful bioindicator of stress in honey bees.

THE STRESS PROTEIN RESPONSE

The persistence of a honey bee colony depends on the survival of individual bees using organismal and cellular-molecular mechanisms to adapt when hive conditions stray from a point of homeostasis. The cellular stress protein (heat shock proteins, HSP) response is an important component of the systemic response that provides organismal resistance to stress (reviewed in: Feder & Hofmann 1999). HSP expression suppresses general protein synthesis during periods of stress. Increased concentrations of HSP accompany proteins to preserve their function and prevent formation of toxic protein aggregations. Feder and Hofmann (1999) drew conclusions from the stress protein literature: (1) HSP genes are found within all organisms that have been investigated for their presence, although patterns of expression may vary, (2) expression of stress proteins occurs in stressful environments in nature, (3) stress protein concentrations are correlated with organismal resistance to stress as well as stress intensity, and (4) stress thresholds induce HSP gene expression to vary among different species according to levels of stress naturally experienced by a species. In these respects, honey bees are no different than other species. Individual bees increase stress proteins after exposure to many stressors including heat stress (42℃ for 4 hours), a variety of pollutants, bacterial infections, and natural enemies (Severson *et al*. 1990; Gregorc & Bowen 1999; 2000; Gregorc *et al.* 2004; 2007; Lipinski *et al*. 2005; Scharlaken *et al*. 2008).

There are six HSP gene families, ranging from small (10-27 kD) to large (90-110 kD), contributing differently to stress responses (reviewed in: Feder & Hofmann 1999). While small HSPs play an important role in cellular tolerance of stress, two gene families (70 and 90 kD HSP) provide stress tolerance at multiple levels of organization (cellular, tissue, and organismal) and regulate the organismal stress response. Severson *et al*. (1990)

compared the heat shock response, increased HSP gene expression, among 0, 9, and 27 day-old worker honey bees following four-hour exposure to 42℃ temperature. Expression of all six gene families increased following heat shock treatment, although age affected HSP expression. The 70 kD stress proteins (HSP70) were present before heat shock and were elevated by heat stress in the 42℃ treatment; smaller stress proteins were not evident prior to heat shock.

Presence of 70 kD stress proteins before and after heat shock is consistent with a multigenic origin of these proteins. Some 70 kD stress proteins (heat shock cognate 70: HSC70) are expressed continuously ("constitutively") and are involved in protein synthesis during normal cell function. On the other hand, inducible 70 kD stress protein (heat shock protein 70: HSP70) expression is triggered only by stressors. The stress protein response may involve increased expression of one (either HSC70 or HSP70) or both (HSC70 and HSP70) proteins. While induction of stress proteins by sublethal stress confers tolerance to higher levels of the same stress and even provides cross-tolerance to other stressors (Feder *et al*. 1997; Krebs & Feder 1997; Krebs *et al*. 2003), we were unable to find studies testing these relationships in honey bees. Evidence that stress proteins play an important role in the stress response of honey bees is provided by a comparative study of brain gene expression in honey bees (Sen Sarma *et al*. 2007). This microarray study of brain tissues in *Apis mellifera*, *A. cerana*, *A. florea*, and *A. dorsata* showed that differential expression of several 70 kD and 80 kD stress proteins contribute to species-specific stress tolerances (Sen Sarma *et al.* 2007).

While increased stress protein expression may improve survival under environmental stress, it may also introduce developmental or physiological costs that are observed long after stressful conditions pass. Studies of the fruit fly *Drosophila melanogaster* demonstrate how larval heat stress alters function of physiological systems crucial to individual performance and fitness. For example, inducible HSP70 expression during heat shock restricts nervous tissue development, affecting nervous system control in fruit flies (Feder *et al*. 1997). Similarly, heat stress during larval development impairs fruit fly locomotor performance in adults by disrupting wing development and weakening tissues involved in walking (Krebs *et al*. 2003). In honeybees (*A. mellifera*) heat stress introduced neural deficiencies that decreased short-term memory and impaired the ability of adult bees to locate food (Jones *et al*. 2005). Since over-expression of stress proteins may be detrimental to an organism, gene regulatory mechanisms must carefully match the degree of the heat shock response with the level of environmental stress.

Heat shock proteins and their transcription factor, heat shock factor-1 (HSF-1), participate with caspases (proteases that are important in cell death or "apoptosis") in regulatory cascades to regulate stress protein expression and cell death (reviewed in: Morimoto 1998; Beere 2004). The stimulus for the stress response is generally accepted to be the accumulation of non-native proteins in the cytosol of the cell. Inactive HSF-1 monomers bound to HSP90 are released when HSP90 binds denatured proteins. HSF-1 monomers assemble into active HSF-1 trimers and then HSP70 and heat shock factor binding protein (HSFBP-1) regulate the translocation of these HSF-1trimers into the nucleus by releasing inactive monomers. In the nucleus, phosphorylation activates HSF-1 trimers which bind to heat shock promoter elements (HSE), activating HSP expression. Translocation of HSF-1 to the nucleus coincides with the appearnance of HSF granules that can be confirmed by immunofluorescent staining.

Increased HSP expression suppresses synthesis of nascent peptides that may misfold under stressful conditions while preserving intermediate folded states of incipient proteins until they can be either refolded or degraded. At sufficient levels, stress proteins (particularly HSP70 and some small HSPs) permit cells to survive stressful conditions because they prevent accumulation of misfolded proteins, escort denatured proteins to proteosomes where protein degradation occurs, and inhibit the caspase-induced apoptosis pathway. When stress conditions overwhelm the cellular protection afforded by stress proteins, inhibition of apoptosis is removed and cell death occurs as a means to remove damaged cells and avoid inflammation (Beere 2004).

An intriguing genetic factor linked to the heat shock response along with HSF-1 is the forkhead transcription factor *Daf-16*, involved in formation of dauer larva formation in the roundworm *Caenorhabditis elegans* (Hsu *et al*. 2003). *Daf-16* is a member of the insulin/insulin-like growth factor-1 (IGF-1) pathway, which is reduced by the insulin-

like receptor, *Daf-2* (Baumeister *et al*. 2006 for review). *Daf-16* has been implicated as a key regulator of heat and oxidative stress resistance, metabolism and developmental arrest (diapause), all linked to stress response and longevity (Finch & Ruvkun 2001). Hsu *et al*. (2003) demonstrated that these two factors were crucial to survival/longevity in *C. elegans*, but were not absolutely required for each other's activities. However, they did find that both factors were required for expression of several small heat shock protein genes (e.g., *Hsp-16.1*, *Hsp-16.49*, *Hsp-12.6*). When interference RNA (RNAi) blocks these genes, the longevity of *C. elegans* over-expressing HSF-1 and *Daf-2* mutants was decreased. This suggests they are directly linked to survival. Another interesting connection between *Hsf-1* and *Daf-16* reported by Singh & Aballay (2006) is that both factors appear to play a role in immune resistance to pathogens in *C. elegans*. They found that *Hsf-1* RNAi decreased resistance to pathogens in animals with a *Daf-2* mutation or over-expression of *Daf-16*.

EXPOSURE OF HONEY BEES TO AGROCHEMICALS

Honey bees contribute substantially to the pollination of various wild plants and food crops. The annual value of agricultural crops benefiting from honey bee pollination is estimated at as much as $20 billion/year in the United States alone (*American Bee Journal*, 1997; Southwick & Southwick 1992). Because honey bees of economic importance live close to and forage within agricultural plots, studying the influence of agrochemicals on honey bee behavior is important for the survival of honey bees, public policy issues, honey bee population regulation, environmental degradation, and the use of biological controls.

The use of toxic chemicals to control insect pests has a long history. Chemicals such as DDT, sevin, rotenon, diazionon, methoxychlor, imidacloprid have been used to control such pests as Colorado potato beetle, cabbageworm, and the gypsy moth. What has not always been known is how these chemicals affect honey bee behavior. Data generated over the past 50 years have shown that pesticides disrupt the functioning of the central nervous system, metabolic processes and some physiological processes such as molting and reproduction. Pesticides which are specially formulated to kill target insects usually do so by influencing receptor molecules in central nervous system, mechanical, photo, and/or chemical receptors. Pesticides have also been developed that are synthetic analogs of enzyme substrates that interfere with metabolic pathways (Winston 1997).

As a case study let us consider the Africanized honey bee in Brazil. The Africanized bee is important to the economy of Brazil in two main ways. Aside from the production of honey as a major agricultural product, bees serve as pollinators of the cotton crop as well as many other crops in the Brazilian economy (*Cotton*, 1997).

Cotton is an important crop for the agrarian sector and development of the textile industry in Brazil. Cotton production in Brazil was adversely affected soon after the appearance of the cotton boll weevil in 1983 and has led, for example, to unemployment, depreciated land value, and the closing of cotton gins and oil mills (Sobrinho & Lukefahr 1983; Ramalho & Santos, 1996). The major strategy to combat the boll weevil is the use of pesticides. The use of pesticides such as endosulfan, decis, baytroid, and sevin to control the boll weevil has adverse effects on the honey bee population. When bees were exposed to baytroid and sevin death quickly resulted. Interestingly, bees exposed to endosulfan acquired a learned response, but over the course of training, the learning became unstable and soon disappeared (Abramson *et al*. 1999). Those exposed to decis showed a pattern of learning indistinguishable from untreated controls.

The study of toxic chemicals on honey bee behavior has extended to the area of sublethal effects. When a toxic chemical is released into the environment it can be diluted, for example, by rain, or degraded by ultraviolet rays from the sun. The result is that honey bees can be exposed to sublethal levels of agrochemicals that normally would be lethal. Evidence exists that sublethal doses of pesticides may be decreasing the number of honey bee colonies available for pollination and reducing the effectiveness of honey bees as pollinators. Sublethal doses of deltamethrin, for example, disrupt the homing flight of honey bees, while parathion disrupts the communication dance of foragers (Schricker & Stephen 1970; Vandame *et al*. 1995). In addition to the disruption of natural behavior, it is known that sublethal exposure to permethrin, coumaphos, and diazinon retards

learning (Taylor *et al*. 1987; Mamood & Waller 1990; Weick & Thorn 2002).

Recently, a new line of investigation has begun on agrochemicals considered harmless to honey bees. These compounds may include some of the new generation pyrethroids, insect growth regulators, and metabolite by-products, all of which are currently used in formulation of new products. Many of these new products are considered by the Environmental Protection Agency, and other regulatory bodies, as user-friendly, target-specific and environmentally safe. However, little is known about their effects, if any, on honey bee behavior. In order to use these chemicals effectively and without injuring these important pollinators it is necessary to know what effects these agrochemicals have on honey bee behavior.

The first experiments on the study of chemicals considered "not harmful" to honey bees was an investigation of dicofol (Stone *et al*. 1997). Dicofol is a chlorinated hydrocarbon pesticide. It is considered nontoxic to most insects and is used primarily to control mites. Honey bees pretreated, however, with dicofol exhibited significantly lower levels of learning than honey bees not pretreated.

Recently, other experiments have been conducted using insect the growth regulators tebufenozide and diflubenzuron (Abramson *et al*. 2004). The results of these experiments were similar to those with dicofol and equally unexpected. The learning ability of honey bees was again disrupted by agrochemicals once thought to be harmless.

As another example let us consider imidacloprid. Imidacloprid is a novel insecticide that mimics nicotine. It is applied to the seeds of crops, and as the plant develops, is transported to the stem and leaves of the plant. Aphids and other pests such as the Colorado potato beetle will die if they ingest imidacloprid. Imidacloprid is also used on sunflower seeds. Sunflowers are an excellent source of nectar for honey bees and sunflowers depend upon bees for pollination. Although it is toxic to honey bees, honey bees are not in direct contact with imidacloprid. It is known from the plant data that the average values of imidacloprid contained in the pollen of sunflowers and of corn was found to be around 3 parts per billion, which is one fifth of the dose known to cause changes in waggle dance communication in honey bees. The French government decided to prohibit use of imidaclopride on sunflower seeds because of its effect on honey bees.

Pesticides are the primary weapon against insect pests (Winston 1997). Unless carefully monitored, the use of agrochemicals can be ecologically unsound, leading to problems such as insect pest resistance, outbreaks of secondary pests, adverse effects on nontarget organisms, pesticide residues, and direct hazards to those individuals applying the chemicals (Devillers & Pham-Delegue 2002). We predict that stress proteins will be useful bioindicators of pesticide-induced stress in honey bees.

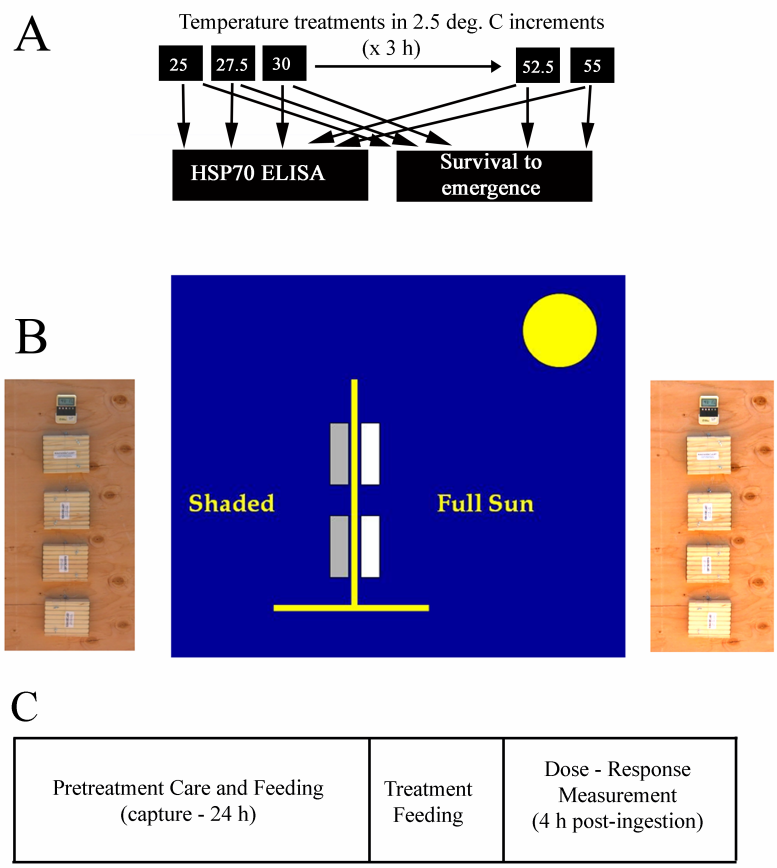
CURRENT APPLICATIONS OF HSP70 TO MEASURE STRESS

Because the correlation between environmental stress intensity, HSP70 expression, and even subcellular HSF-1 and HSP70 localization are well established (reviewed in: Morimoto 1998), numerous studies use concentrations or localization of these molecules as biomarkers of sublethal stress. Quality antibodies from chemical suppliers have been developed to detect conserved HSP70/HSC70 domains and are being used in many organisms to quantify HSP70 family protein levels following stress. Densitometry of Western blots and enzyme-linked immunoassays (ELISA) have been used to compare levels of the principal stress protein, HSP70, as a sensitive indicator of sublethal stress in many organisms (*e.g.,* Gibney *et al.* 2001). The HSP70 ELISA is more sensitive than Western blots to subtle changes in HSP70 expression that accompany sublethal stress (*e.g.,* Pempkowiak *et al.* 2001). While other bioindicators (*e.g.*, vitellogenin) may be used to monitor stress responses in specific organ systems, HSP70 concentrations are useful as a general bioindicator of stress that is sensitive to many biotic and abiotic stressors (*e.g*., Maradonna *et al*. 2007).

With the goal of monitoring how stress impacts honey bee populations, measurement of individual stress protein responses should be useful for evaluating management practices, seasonal changes, and sublethal effects of agrochemicals. Toxicological studies investigating the effects of numerous chemical stressors on honey bees have used subcellular localization of HSF granules in the nucleus as an indicator of stress (Gregorc & Bowen 1999; 2000; Gregorc *et al*. 2004; 2007). Studies over the last eight years have used ELISA to monitor quantitative changes in 70 kD stress

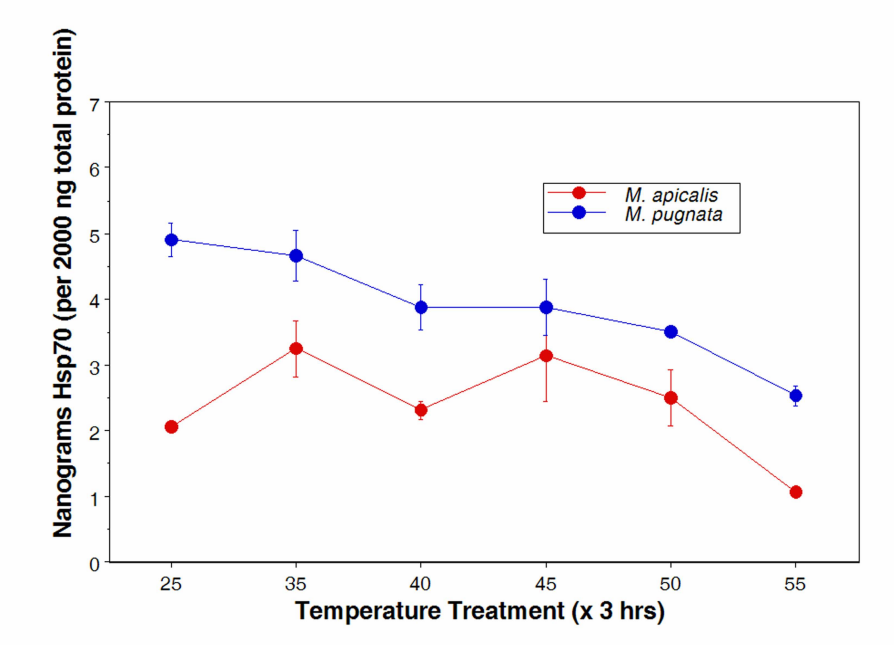
protein concentrations and Western blots to detect expression of constitutive and inducible HSP70 gene family proteins in bees. Three different experimental designs were used in our studies of stress responses in bees (Fig. 1). Using these techniques, differences in the larval/prepupal thermotolerance and survival of two species of leafcutting bee (*Megachile rotundata* versus *M. apicalis*) were detected in laboratory experiments (Fig. 1A; Barthell *et al*. 2002). These techniques also revealed developmental changes in HSP70 concentrations associated with emergence from diapause in *M. rotundata* (Hranitz and Barthell 2003). In field studies of *M. apicalis*, HSP70 concentrations revealed abiotic stress in all life stages (egg through adult) in a nesting habitat used more by exotic solitary cavity-nesting bees than native bees (Fig. 1B; Barthell *et al*. 1998; Hranitz *et al*. 2009). Laboratory studies of honey bees also used the HSP70 ELISA to compare pretreatment stress to ethanol-induced stress four hours after ingestion (Fig. 1C).

Figure 1. An overview of several experimental designs used to measure stress responses in bees. (A) Groups of solitary bee larvae in nest cells were placed in treatments in a temperature gradient and then either sampled for the HSP70 ELISA or allowed to develop through emergence for estimates of mortality rates (Barthell *et al*. 2002). (B) Field experiments conducted on the leafcutting bee *Megachile apicalis*) used north- and south-facing trap nests to measure HSP70 concentrations and mortality rates after exposure to abiotic stress under field conditions (Hranitz *et al*. 2009). (C) Laboratory experiments compared stress responses of honey bees during pretreatment care and feeding and 4 h after being fed sucrose (control) or several doses of ethanol.



To demonstrate how HSP70 concentrations may reveal levels of stress among treatments, we present a comparison of the stress responses of two leafcutting bees, *Megachile pugnata* (native to the USA) versus *M. apicalis*, which is invasive to the western USA. Larvae in nest cells were acclimated to room temperature for several days to break diapause. Nest cells were divided into groups that were exposed to different temperatures for 3 hours (the duration of high temperatures during a typical afternoon in Central California) as indicated in Fig 1A. At all temperatures, the native leafcutting bee demonstrated higher HSP70 concentrations than the invasive leafcutting bee (Fig. 2). The higher stress response of *M. pugnata* indicates a lower larval tolerance of high temperatures than the larvae of the invasive *M.apicalis*.

Figure 2. Stress responses of prepupal larvae by the native (USA) leafcutting bee *Megachile pugnata* and the invasive (USA) leafcutting bee *M. apicalis*. HSP70 concentrations (mean ±SEM) were higher at all temperature treatments for *M. pugnata* relative to *M. apicalis*.



Recently, Williams *et al*. (2008) showed that HSP70 expression differs with flight activity in nurse bees and, in foragers, was higher in flight muscle than brain tissue. Increased HSP70 concentrations respond to the rapid accumulation of reactive oxygen species (ROS) associated with flight. The antioxidant protection of these tissues by HSP70 and other mechanisms decreases with age. Most recently, we used HSP70 concentrations in honey bee brain tissues to monitor the effects of pretreatment handling, typical for isolating individual bees during feeding stimulus studies on stress in honey bees (Hranitz and Abramson, unpublished manuscript). Ethanol metabolism, similar to exercise stress, produces a variety of ROS that lead to oxidative stress. In one study (Hranitz and Abramson, unpublished manuscript), pretreatment handling procedures for honey bees did not significantly increase HSP70 but we observed a positive dose-dependent stress response among the alcohol treatments. These studies demonstrate how the HSP70 ELISA may be used to the measure sublethal stress intensity.

FUTURE STUDIES OF THE STRESS RESPONSE IN HONEY BEES?

While still ongoing, the Honey Bee Genome Project has already produced microarrays to screen brain tissue for altered expression of thousands of honey bee genes, including many stress protein genes and their regulatory proteins (Whitfield *et al*. 2003, 2006). Annotation of the complete honey bee genome will eventually characterize the six gene families and their regulatory protein genes. However, for practical reasons, the prospect for large-scale colony monitoring that would reveal whether sublethal stress influences honey bee hive performance is currently low. Microarrays are costly and yield more information than needed to measure whether or not environmental factors are stressful to honey bees. Analysis of microarrays also requires technical expertise to gather and interpret results. Compared to microarrays, ELISA is relatively inexpensive, requires technical expertise easily gained through undergraduate education, and is well suited for rigorous experimental designs to test hypotheses using parametric statistics (*e.g*. Barthell *et al*. 2002; Hranitz *et al*. 2009).

The aforementioned studies of honey bees and solitary bees suggest a range of research opportunities yet to be explored in studies relating to honey bees. Better understanding of sublethal stress phenology (*e.g*. seasonality in temperature, availability of floral resources, natural enemies, transport of bees, general beekeeping management practices such as inspection of bees and, very often, use of smokersat hives) may alert bee keepers to needed changes in hive management practices. Timing of stresses may have important influence on hive resources or susceptibility to natural enemies. Sublethal stresses, particularly chemicals (pesticides) that may be introduced into the hive, may not kill adult bees outright but instead impair worker, queen, or drone performance and thereby reduce colony performance. Developmental exposure to sublethal stress can also substantially decrease fitness or performance traits as demonstrated for locomotor performance in *D. melanogaster* as well as learning and memory in honey bees. These avenues of stress protein research offer relatively unexplored avenues of research that may contribute important knowledge of honey bee performance in natural and agricultural environments.

ACKNOWLEDGEMENTS

We appreciate our collaborations with the many undergraduate student researchers who participated in our studies in Turkey and the USA. We are especially grateful for the support of Ulüdag University during studies conducted in Turkey. The National Science Foundation's REU Program (DBI #0552717) provided funding for faculty and students in Turkey. We also thank our participant involved in the REU project, I. Çakmak (Uludag University), and S. Çakmak who have provided many wonderful ideas and without whose support our research in Turkey would not have been possible. Lastly, we thank two anonymous referees for helpful reviews and the journal staff for their assistance with the Turkish translation.

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GENİŞLETİLMİŞ ÖZET

Amaç: Bu derlemenin amacı bal arılarında stres proteinlerinin çalışma mekanizması, stres proteinlerinin stres zamanında bal arılarında hücresel-moleküler ve organizma seviyesinde nasıl bir tolerans sağladığı, her bir arının kovanda bir biyo-gösterge olabileceğini tartışmaktır.

Tartışma: Bal arılarında ileri derecede sosyal yapı çevresel faktörlere ve kovandaki dengelerin kurulmasında koloni işbölümü

sayesinde çok geniş bir tepki olanağı sağlamaktadır. Işçi arılar tarafından sağlanan kovan dinamikleri koloni kayıp zamanlarındaki strese karşı dengelenmelidir. Stres proteinleri çevredeki çok farklı stres faktörlerine karşı hücresel-moleküler tepki mekanizmasının önemli bir parçasıdır. Bal arısı kolonisi grup olarak çok farklı çevre koşullarında özelleşmiş rekabetçilere karşı rekabet edip yaşamaya devam edebilmesi için kovan içindeki iklimsel dengeyi de korurlar. Bunu yayılmacı arılar nektar yerine su toplayarak buharlaşma ile çok sıcak havalarda kovanda soğutma yaparak sağlar. Nectar stresi bal arılarında çiçeklere yayılma davranışı ve diğer nektar toplayan canlılar arasında bir yarışmanın sonucudur. Bu durumda yayılmacı arılar her ne kadar maksimum net enerji hedefleselerde çiçekleri paylaşımda da bir işbölümü olduğu ve bunun rekabeti azalttığı görülmektedir. Bakteriden insana kadar tanımlanmış ve moleküler hücre stres tepki mekanizmasının bir parçası olan stres proteinleri biyolojik organizasyonda organizma seviyesinde stres faktörlerine karşı koruma sağlamaktadır. Literatürde yaygın stres proteini birçok genel yapı göstermektedir. Ölümcül olmayan stres hücre seviyesinde stres proteinlerinin konsantrasyonunu artırmakta ve arkasından gelecek strese karşı yaşama direncini artırmaktadır. Fakat üretilen stres proteinlerinin canlı açısından bir bedeli bulunmaktadır. Bir taraftan stres koşullarında fazla üretilen stres proteinleri yaşama gücünü artırırken gelişme döneminde yaşamın daha sonraki evrelerinde farklı koşullar altında önemli olan bazı başarı karakterlerinin kaybedilmesine neden olabilir. Bir kaç çalışma stres proteinlerinin cansız faktörlerle (sıcaklık, zehirler) alkol tüketimi ve uçuşla ilişkili oksijen stresi arasındaki ilişkilerini araştırmıştır. Sıcaklık stresi bal arılarında sinir sisteminde bozukluk ve kısa süreli hafızanın kaybedilmesine ve yayılmacı arıların besinin yerini bulmasını engellemektedir. Pestisit olarak zararlı organizmalara karşı kullanılan kimyasallar merkez sinir sistemi fonksiyonu, deri değiştirme ve üreme gibi fizyolojik gelişmeleri bozmaktadır. Elde edilen veriler pestisitlerin ölümcül dozun altında olması durumunda bile tozlaşma için önemli olan kolonideki yayılmacı arı sayısını azalttığnıı göstermektedir. Ek olarak permethrin, coumaphos, diazin gibi kimyasaların bal arılarında öğrenmeyi engellediği bilinmektedir. Son zamanlarda (yeni nesil pyrethroids, böcek büyüne düzenleyicileri gibi) hedefe özel çevreye dost ve güvenli görülen tarımsal kimyasalların bal arılarında davranışı nasıl etkilediği konusunda çok az bilgi bulunmaktadır. Örneğin, Dicofol'un çoğu böceklere karşı zehirli olmadığı kabul edilir fakat bal arılarında kullanıldığı zaman öğrenme seviyesinde önemli derecede kayıp görülmüştür.

Sonuç: Sonuçta böceklere karşı kullanılan kimyasallar tarımsal ilaçlar ekolojik açıdan doğru olmayan, zararlıların direnç geliştrmesine, ikincil zararlıların çok sayıda artmasına, hedef olmayan canlılarda olumsuz etkilere, kalıntı sorunlarına, ve üzerinde uygulanan canlılara zrar vermektedir. Bu bakımdan stres oluşturması nedeniyle benzer şekilde farklı konsantrasyonlarda alkol solüsyonları ile çalışmalar yapılmaktadır. Ölümcül dozun altındaki tarımsal kimysallar kovanda direk olarak arıları öldürmeyebilir, fakat işçi arıları, ana arıyı ve erkek arıları olumsuz etkileyerk kolooninin veriminin azalmasına neden olabilir. Bal arılarının ekonomik önemi (farklı mevsimlerde sıcaklık, besinin durumu, kovanların gezginci arıcılıkta taşınması, kolonilerin rutin kontrolleri, körük kullanımı, koloni bakım-besleme, pestisitler, doğal düşmanlar) nedeni ile tarımsal faktörlerin koloni performansını nasıl etkilediği, her bir arıda başarı karakterleri ve stres tepkileri arasındaki ilişkinin belirlenmesi gelecekte araştırılması gereken bir konudu