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Determination of the Impact of Mating on Stress Protein in Different Honey Bee Breeds

Farklı Bal Arısı Irklarında Çiftleşmenin Stres Proteini Üzerine Etkisinin Belirlenmesi

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Abstract

The queen is the only individual with a colony of bees and the ability to reproduce. This study determined the level of stress proteins (Hsp70) in mated and virgin queens reared under the same conditions in colonies of honey bee breeds and ecotypes in Turkey. When the effects of mating were examined, it was found that the stress protein content of mated queens was significantly lower than that of non-mated queens ($p<0.05$). It was also found that Hsp70 stress protein levels were lower in Thrace, Yığılca and Gökçeada ecotypes, which are the sub-ecotypes of the Anatolian bee, compared to other ecotypes and races. As a result, it was found that the adaptive abilities of Trakya, Gökçeada and Yığılca ecotypes, which are the sub-ecotypes of the Anatolian bee found in our country, are better than those of other subspecies.

Keywords: Honey bee, Queen**, S**ubspecies, Mating effect, Stress protein (Hsp70)

Özet

Ana arı bir koloninin devamlılığını sağlayan ve üreme yeteneği olan tek bireydir. Bu çalışmada Türkiye'de bulunan bal arısı ırk ve ekotiplerine ait kolonilerde aynı koşullarda yetiştirilen çiftleşmiş ve çiftleşmemiş ana arılarda stres proteini (Hsp70) seviyesi belirlenmiştir. Çiftleşmenin etkisine bakıldığında çiftleşmiş ana arıların stres proteini seviyesi çiftleşmemişlere göre önemli derecede daha düşük olduğu belirlenmiştir ($p<0,05$). Ayrıca yetiştirme koşullarında Anadolu arısının alt ekotipleri olan Trakya, Yığılca ve Gökçeada ekotiplerde Hsp70 stres proteini seviyesinin diğer ekotip ve ırklara göre daha düşük olduğu tespit edilmiştir. Sonuç olarak ülkemizde bulunan Anadolu arısının alt ekotipleri olan Trakya, Gökçeada ve Yığılca ekotiplerinin adaptasyon yeteneklerinin diğer alt türlere göre daha iyi olduğu belirlenmiştir.

Anahtar Kelimeler: Bal arısı, Ana arı, Irk ve ekotipler, Çiftleşme etkisi, Stres proteini (Hsp70)

1. INTRODUCTION

The world is home to a diverse range of honey bee breeds. These breeds have adapted to the environmental conditions in which they live and display diversity in their morphological, behavioral, and yield features. They have also adapted to the environmental conditions in which they exist. Tens of thousands of years ago, honey bees were already present in the Anatolian region. Because of this, they have differentiated in order to adapt to the diverse environmental conditions. According to the findings of a number of researchers (Bodenheimer, 1942; Fıratlı, 1987; Sonmez & Settar 1987; Ruttner, 1988; Kandemir et al., 2000), the region of Anatolia is home to a wide variety of honey bee ecotypes and breeds. The Anatolian bee, also known as *Apis mellifera anatoliaca*, the *Caucasian* bee, also known as *Apis mellifera caucasica*, the Syrian bee, also known as *Api mellifera syriaca*, and the Carniole bee, also known as *Apis mellifera carnica*, are the five species of *Apis mellifera* (Kandemir et al., 2000). In addition to the five distinguishable bee breeds that have already been mentioned, regional ecotypes of bees that are specialized in terms of the morphological and genetic characteristics that they exhibit have also emerged in Turkey as a result of the country's diverse flora and climate structures across its various regions. According to Kekecoglu (2010), some of these ecotypes include the Muğla, Gökceada, native Hatay, Yığılca, Trakya, and Efe ecotypes.

A honeybee colony consists of a queen, tens of thousands of worker bees, and hundreds of drones. The number of worker bees in a colony is what determines its population. This number varies depending on the time of the year when the, a queen's ability to lay eggs, the abundance of nectar and pollen sources, the level of environmental stress, and the presence of parasites and other harmful organisms within the colony. According to Köseoğlu et al., (2017), the only individual capable of maintaining a colony as well as having the ability to breed is the queen bee. The quality of queen bees can be affected by a variety of circumstances. These factors include the selection of the genotype, the supply of breeding material, the breeding method, the status of the starter colony, the age and number of larvae, the breeding season, the number of drones, and the nutritional status (Şahinler & Kaftanoğlu, 1997; Doğarolu, 2004; Cengiz et al., 2019; Arslan & Cengiz, 2020; Arslan et al., 2020). Doğarolu (2004) and Şahinler & Kaftanoğlu (1997), list these factors in their respective works. Honey bees are one of the most beneficial insects on Earth with both their critical roles in pollination and health-promoting products (Oskay et al., 2023). There have been few studies conducted on the subject of the link between the effects of agricultural factors on colony performance and stress reactions (Hranitz et al., 2009). Climate change is one of the most important issues in the 21st century. As a result of the increase in temperature, various changes in our climate may occur, such as changing precipitation distribution and the frequency of severe weather events. It is estimated that global warming will raise sea levels by several meters by the end of this century, and hurricanes and heat waves will be more frequent than now (Barth & Titus, 1984; Adediran et al., 2023; Oskay & Sönmez, 2023). According to many researchers, climate change is considered dangerous for many living species and biodiversity (Mahakunda & Tiwari, 2024). The first requirement for productive and profitable beekeeping is to deal with robust colonies that are led by queen bees that are still young and of excellent quality. When compared to weak colonies managed by inadequate and low-quality queens, productive output per colony is significantly higher in robust colonies managed by quality queens (Öztürk, 2014). This is something that should be taken into consideration. It is necessary for queens to mate in order to ensure the development of the colony and the continuation of production. Bees experience both short-term and long-term changes in their physiology and behavior as a result of mating. On the other hand, not a lot of research has been done to investigate the molecular pathways that are responsible for these post-mating alterations (Kocher et al., 2008). After mating, the queen's pheromone profile as well as the size of her ovaries and the maturity of her eggs undergo dramatic alterations (Plettner et al., 1997; Tanaka et al., 2004; Richard et al., 2007). Dopamine and N-acetyldopamine levels fell after mating while this was all occurring (Fahrbach et al., 1995; Harano et al., 2005).

Excessive secretion of the hormone dopamine is known to result in a stress reaction (Harris & Woodring, 1992). HSP 70 is the most widely utilized biomarker for monitoring honey bee stress. Hsp70, one of the heat shock proteins, functions as a molecular chaperone to protect cells against the deleterious consequences of protein denaturation under unfavourable circumstances. The stress protein (Hsp 70) is a system reaction that supplies the organism's stress resistance (Feder & Hofmann, 1999). Under unfavorable conditions, the expression of

Hsp70 (Ashburner, 1982), which has been documented in several animal taxa, including insects, promotes cell survival and tolerance to stress. Due to the division of work within the colony in creating the equilibrium of the hive and external influences, the complex social structure of honey bees offers a broad variety of reaction options. The worker bees' contribution to the dynamics of the hive must be balanced against the strain of colony loss periods. Stress proteins are a crucial component of the cellular-molecular response system to several environmental stressors. (Hranitz et al., 2019) The honey bee colony maintains the hive's climatic equilibrium as a collective in order to compete and thrive against specialized rivals in vastly varied environmental circumstances. As can be seen, bees employ a variety of defense systems to mitigate the damage that environmental stress might produce (Goulson et al., 2015; Li et al., 2018). Numerous biotic and abiotic stressors, including diseases (parasites, fungus, viruses, and bacteria), ecosystem changes or losses, and the use of agrochemicals, have a detrimental impact on the health and lifespan of the bee colony, either individually or in combination. All of these elements alter the bees' immune system and defensive systems (Bruutcher et al., 2015; Li et al., 2018; Larsen et al., 2019). In recent years, a number of researchers have been conducted on the origins of stress and possible preventative measures for honey bees; improper colony management, bee disease and pests, frequent colony transfers, rapid climatic changes, and flora deficit are just a few examples (Topal et al., 2019).

In this work, we assessed, for the first time, the effects of adaptation to environmental circumstances and mating on stress protein (Hsp 70) in queens generated from several honey bee subspecies and ecotypes maintained under identical conditions.

2. MATERIALS and METHOD

2.1. Queen Bees Breeding

Queen bees of different breeds and ecotypes used in the study obtained from Directorate of Aegean Agricultural Research Institute Efe bee ecotype; Macahel Beekeeping Caucasian Camili ecotype; Macahel Beekeeping Caucasian Posof ecotype; Muğla Beekeepers Association Muğla bee ecotype, Kırklareli Beekeepers Association Trakya bee ecotype; Erdoğan Çıracı Queen Bee Enterprise Yığılca ecotype, Gökçeada Beekeepers Association Gökçeada bee ecotype, Macahel Beekeeping Anatolian bee breed, Hatay Mustafa Kemal University Hatay bee breed. These queen bees were kept in the town of Ordu, Perşembe during the whole study.

Every three days, the breeding materials that were employed in the study were given sugar syrup at a ratio of 1:1 as part of their diet. A random sampling was carried out after the queen bees that had returned from their mating flight had started to lay eggs and had sealed their chambers. Dissection was carried out on virgin queen bees that had just emerged and the bees were taken to the laboratory immediately.

2.2. Stress Protein (Hsp 70) Analysis

Queens of different honey bee Subspecias and ecotypes were brought under Ordu conditions and included in nucleus colonies. Queens were bred from these breeding colonies and mated with them, and the virgin queens were taken to the laboratory and kept in a freezer at -20 °C until the bee brains were removed.

Brain tissue was extracted from bee samples for study using a microscope and dry ice and then transferred to centrifuge tubes. The brain tissue in these tubes was homogenized and extensively crushed using PBS-azide-TAME buffer. Following centrifugation at 13,000 x g for 20 minutes at 4°C, total protein concentrations in the supernatant were determined using a protein assay kit (#5000112, Bio-Rad, Hercules, CA, USA). According to the standards (Hranitz et al. 2010; Güneş et al. 2017), the HSP 70 values in 2000 ng total protein were read, the appropriate dilutions were produced according to the total protein values, and the concentrations of the samples were determined. We utilized H5147-Sigma-Aldrich Monoclonal Anti-Heat Shock Protein 70 antibody as the primary antibody for coated Eliza, while our secondary antibody is Goat Anti-Mouse IgG (H + L)-HRP Conjugate #1706516 (Hranitz et al., 2010; Güneş et al., 2017; Sarıoglu –Bozkurt et al., 2022).

2.3. Statistical Analysis

Using the IBM SPSS Statistics 23 application, the gathered data were categorized by bee species. The data's normality was evaluated and determined to be less than $P=0.05$. Since the non-normal data were statistically evaluated with the non-parametric Kruskal- Wallis test and there were nine distinct groups, mean values and standard deviations were calculated.

3. RESULTS and DISCUSSION

In accordance with the reported Hsp-70 levels, virgin bees were observed to be more stressed than virgin bees in general. When comparing mated and virgin bees within the Yığılca Bee, Caucasian Posof Bee, and Caucasian Camili Bee populations, $p<0.05$ was shown to be statistically significant (Table 1). ($P<0.05$ was found to be significant between the different letters in the different columns.

In all breeds, the pre-breeding values were greater than the post-breeding values. Statistically significant, however, are just three bee breeds. Trakya, Yığılca, and Gokceada ecotypes, which are subecotypes of the Anatolian bee, have a lower Hsp70 stress protein concentration, as revealed by the research. During the study, it was revealed that queens from these breeds and ecotypes had shorter queen acceptance rate, mating flight, and egg-laying periods than queens from other breeds and ecotypes, as well as fewer aggressive tendencies. The Caucasian breed has a higher queen acceptance rate, longer mating flight duration, and longer egg-laying period compared to other breeds.

Table 1. According to bee breeds, the HSP 70 levels of mated and virgin bees were evaluated

Bee breed	**HSP-70 (ng/ul)**	
	Virgin	Mating
Muğla Bee	19.20 ± 2.76	18.86 ± 2.83
Anatoliaca Bee	19.95 ± 2.72	18.24 ± 2.98
Hatay Bee	23.32 ± 2.21	18,85 ± 2,34
Gökçeada Bee	19.79 ± 2.95	12.48 ± 2.15
Efe Bee	21.08 ± 2.66	15.86 ± 2.76
Trakya Bee	14.61 ± 2.92	8.15 ± 2.36
Yığılca Bee	23.96 ± 1.19[a]	10.34 ± 2.74[b]
Causica Posof Bee	23.77 ± 2.05[a]	14.92 ± 1.01[b]
Causica Camili Bee	23.21±2.77[a]	15.14 ± 2.26[b]

Although not statistically significant, the Trakya Bee was found to have the lowest Hsp70 levels both before and after mating. This is the most ideal bee for adapting to the circumstances of the Ordu province. When our findings are analyzed, it is possible to conclude that the statistically significant bees can adjust to their new environment more readily than other varieties. It was discovered by Sarioglu et al. (2021) that the stress reaction of queenless colonies was greater than that of queenright colonies. According to the same study, the stress levels of the starting bees given sugar syrup were greater than those of the finisher bees. The low Hsp70 values in bees corroborate our findings that dopamine levels decline after mating, as demonstrated by research (Harris & Woodring, 1992) (Figure 1).

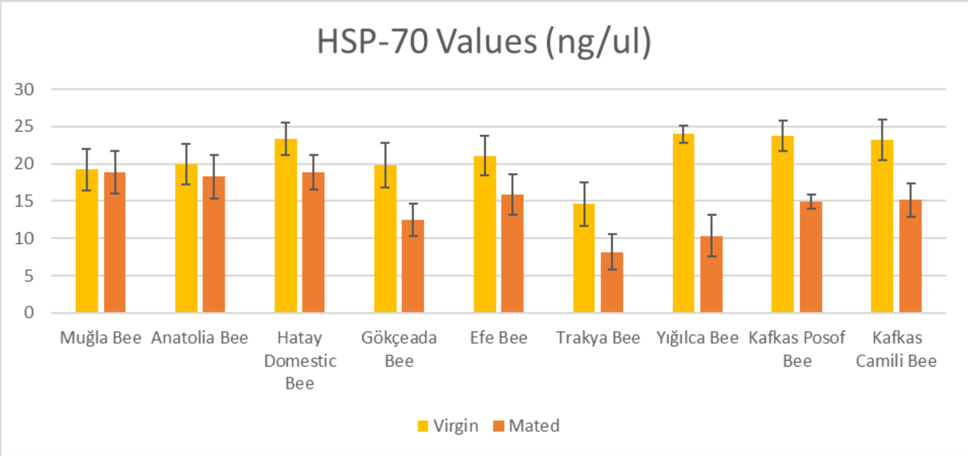

Figure 1. Differences between the HSP 70 values of mated and virgin queen according to mating queen

4. CONCLUSIONS

As a result of its diverse climate and flora, Turkey is a rich source of racial ecotypes for bee genes. In terms of being the mother of all members of the colony, the queen bee is the most important individual in the honeybee colony. All genetic features (chitin color, disease resistance, swarming, etc.) shown by a colony are due to the genes of the queen. Having queen bees of excellent quality in the colonies is a need for the desired efficiency/benefit in beekeeping. In this study, various breeds and ecotypes of queen bees from Turkey were maintained in Ordu. The environmental adaptability capabilities of these queens and the effect of mating on Hsp 70 stress protein were determined. According to the findings of the study, it was discovered that the ecotypes acclimated to varied geographical circumstances, and not the queen bees of different breeds, had superior adaptation capacities. In all breeds and ecotypes, Hsp70 stress protein levels were found to be lower in mated queens. As a result of the investigation, it was discovered that honey bee ecotypes in our nation had superior adaptability. The levels of stress protein (Hsp70) in Trakya, Yığılca, and Gokceada ecotypes were determined to be (8.15 ± 2.36), (10.34±2.74) and (12.48±2.14), respectively.

DECLARATIONS

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

The author contributes the study on his/her own.

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