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Relationships between hygienic and grooming behaviour in Yığılca honey bee (*Apis mellifera* L.) Ecotype

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Abstract
The aim of the study to investigate the relationships between hygienic (HB) and grooming (GB) behaviours on Yığılca honey bee colonies which is known to have rapid colonial development before main nectar flow and mid-range resistance to Varroa destructor. The hygienic behaviour of colonies was initially evaluated with a well-tested assay utilizing pin-killed brood. Subsequently, grooming behaviour was evaluated using the natural fall of Varroa mites. It was observed, negative and positive linear correlations emerged subsequently from early spring to end of the summer. Results of the experiments showed that the correlations occur depend on seasonal periods. This is the first study in which HB and GB were evaluated for Anatolian honey bee.

Keywords: Hygienic, grooming, behaviour, Yığılca honey bee

1. Introduction

Apis mellifera L. is in worldwide danger because of the ectoparasitic mite *Varroa destructor* (Bradbear, 1988; Matheson, 1996; Anderson & Trueman, 2000) [9, 2, 21]. *A. mellifera* colonies commonly die because of the *V. destructor* infestation within a few years if colonies are not treated (Rosenkranz and Engels, 1985; Ritter, 1988) [26, 25]. Various strategies have been suggested to control of *V. destructor* including physical and chemical methods (Le Conte *et al.*, 2010) [17]. Chemical acaricides are extensively - used to control *V. destructor* because it takes less effort and less time - than alternative methods. However, many of these pesticides cannot be used when hives are being used to produce honey. Also, resistant strains of *Varroa* mites can develop due to wrong chemical applications (Spreafico *et al.*, 2001) [28].

Thus, the development of non- chemical, sustainable mite management methods are desirable to avoid pesticide resistance to and to prevent contamination of bee products. One strategy is to breed bees with a higher tolerance and resistance to varroa mite. Hygienic behaviour is the main natural defence against brood diseases of the honey bee (Spivak & Gilliam, 1998a) [27], and it is also a natural defence against *V. destructor* mites infesting brood cells (Boecking & Spivak, 1999) [8]. It might contribute to overall resistance against *V. destructor* as a behavioral trait of the bees. In hygienic colonies worker bees detect, uncap, and remove infested, diseased, or parasitized broods from the nest thereby limiting disease transmission (Boecking & Drescher, 1991) [7].

In addition to hygienic behaviour, there are several other known resistance mechanisms against *V. destructor* including grooming behaviour, which adult bees remove *Varroa* mites from other bees, meanwhile damaging the mites (Boecking & Spivak, 1999) [8]; yet unknown physiological characteristic of pupae or adult bees in some colonies which reduces mite reproduction (Harbo & Harris, 1999) [13]. Commercial lines of honey bees with resistance to *V. destructor* may moderate the need for expensive and frequent acaricide use.

Other strategies based on treatment of the physical or mechanical methods are shown to be useful in *Varroa* mite management. One of the most successful of these has been the use of hive bottom boards (Pfefferle, 1980; Anon, 1993) [24, 4]. Mesh bottom boards use a metal mesh which aims to cause the *Varroa* mites to fall out of the hive structure, directly reducing in-hive populations (Ellis & Delaplane, 2001; Pettis & Shimanuki, 1988) [10, 23].

However, reported results of its efficiency have been inconsistent, mainly because its success depends on the level of hygienic behaviour of the treated colonies. For this reason, the best application for management is to use *Varroa* resistant honey bee lines.

In the present study we aimed to reveal potential HB and GB behaviour with Yığılca honey bee ecotype regarding to season. As a local ecotype, Yığılca honey bee diverged from their origin of Central Anatolia (*A. m. anatoliaca*), and adopt to shorter vegetation period and mild temperate zone in the Black Sea Region. This honey bee ecotype starts to produce more broods before the main nectar flow and had a larger worker bee population during the period of nectar flow (Gösterit *et al.*, 2016) [11]. Therefore it was hypothesized that ıf there are changeable relations between HB and GB behaviours according to season. This is the first study in which HB and GB were evaluated for Anatolian honey bee.

2. Material and Methods

2.1 Evaluation of hygienic behaviour at 0. and 24. hour

The study was conducted in Düzce University Beekeeping Research Development and Aplication Centre, Düzce/Turkey. To screen experimental colonies, 40 colonies of Yığılca honey bee ecotype were tested for hygienic behaviour using pin- killed brood assay:

In this study we used pin-killing method to 40 colonies to determine the performance of hygienic behaviour of the bees located in the Yığılca district. The first day, 3 honeycombs with capped cells were removed from each hives. 100 capped brood cells comprising 10 to 14 days old pupa were perforated with the pin-killing method. The test section was also mapped on plastic sheets to simplify identifying them. We made a hole in the brood cells, then a 6.5 cm diameter circular section of capped worker brood enclosing approximately 100 cells. Test sections were photographed by a digital camera (Fig. 1). The combs with the perforated brood cell placed back into the center of the brood nest of their colonies. After removed from colony the test sections were photographed within 24 hours. The combs were observed two times, until 24 hours after brood cell perforation. The observation was made by following characters: number of capped cells, number of empty cell, number of punctured cells, number of uncapped cell, number of cell with brood partially removed (Kekeçoğlu *et al.*, 2015) [16].

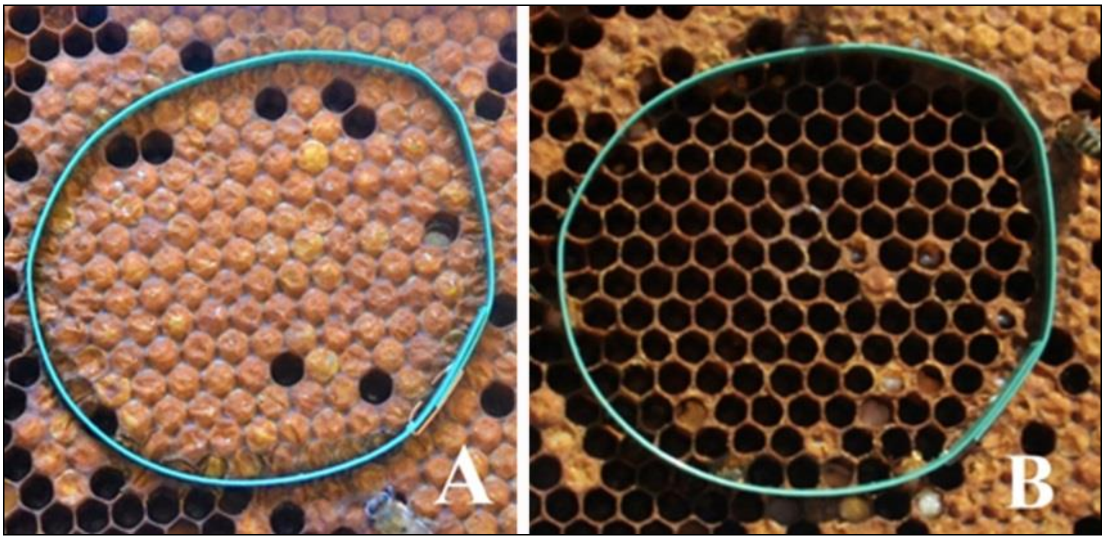

Fig 1: Hygienic behaviour performance; pin-killing test at 0. hour (A) and 24. hour (B)

2.2 Evaluation of grooming behaviour

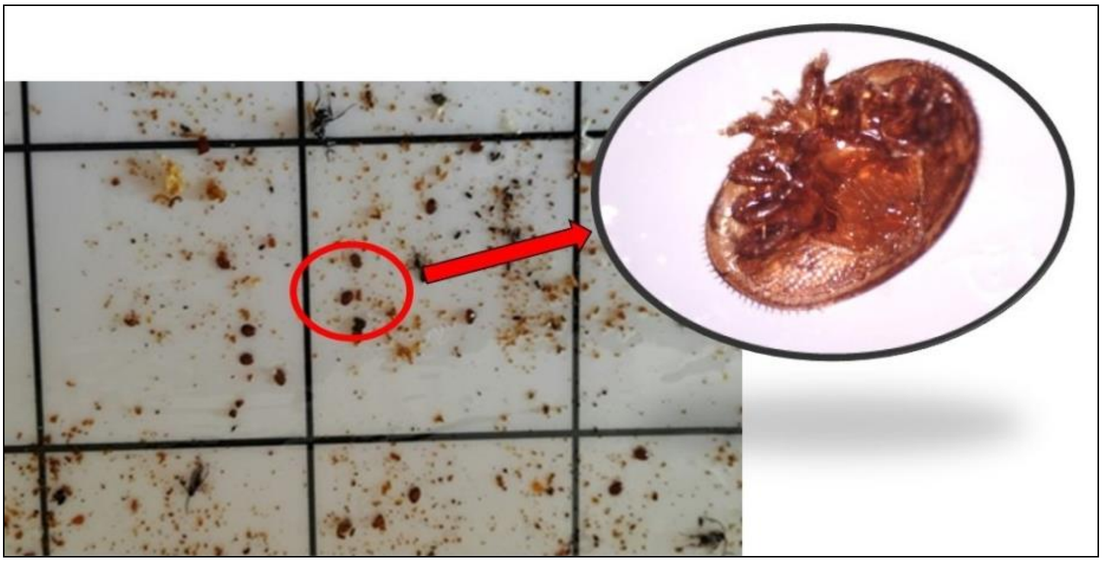

Fig 2: Special board papers and *Varroa destructor* samples

To evaluate grooming behaviour, the slightly modified methodology was applied (Martin *et al.*, 1998) [20]. It was used a special board papers underneath the colony with bottom board (Fig. 2). Data were collected for one week to figure obtain mite fall average daily. This period covers natural variation in mite fall due to population dynamics cycles within the host and calculated the total colony infestation rate from the weekly might fall by multiplying the daily mite drop by 250–500 or 20–40 when brood is absent or present, respectively.

2.3. Statistical analysis
Descriptive statistics were calculated and normality tests were applied to performance data (Table 1). Nonparametric correlation test (Spearmen R) were used to analyze relationships between HB and GB variables in pairs of performance groups formed by months. Correlation statistics were showed as associated with scatterplots for each month (Figs 3-7). Statistical analysis was performed using the Statistica program.

3. Results
This experiment was designed to reveal relationships between hygienic and grooming behaviors, considering the shorter vegetation period, rapid colonial development properties and *Varroa* resist. This experiment was performed to 40 colonies of Yığılca ecotype of *Apis mellifera anatoliaca*, from March to July. Descriptive statistics were summered in table 1 and nonparametric correlation analyze (Spearmen R test) used to analyze obtaining data.

Table 1: Descriptive statistics of HB and GB performances

Min.- Max. Mean±Std.	March n=40	April n=40	May n=40	Jun n=40	July n=40
HB	29,03 - 95,80	8,41 - 95,46	52,53 - 99,16	33,98 - 99,18	43,02 - 95,92
	73,58 ± 18,26	59,35 ± 23,00	79,81 ± 12,64	85,37 ± 13,13	73,71 ± 12,56
GB	0,00 - 74,00	0,00 - 188,00	1,00 - 199,00	0,00 - 366,00	1,00 - 267,00
	12,24 ± 14,99	28,91 ± 34,35	53,51 ± 55,14	55,71 ± 77, 30	52, 53 ± 57, 91

Results of the experiments showed that positive and negative tended correlations can occur within the season. Correlation analysis showed that positive and negative relationships may change suddenly in consecutive months, and the level of relationships in the same direction may increase or decrease further. During the March, June and July, negative linear relationships were detected, while positive linear relationships showed in April and May.

Although negative relationships tended at lower rate, HB-GB correlation value of July (r= -0.2545 P<0.05) was more significant than that of other months (r=-0.0297 P<0.05, r=-0.0129 P<0.05) (Figs 3, 6, 7). In April and May, positive relationships were appeared at low level (r=0.1648 P<0.05; r=0.0932 P<0.05, respectively) (Fig 4. and Fig 5.).

Bioecological observations: In addition, it was observed, population development (worker bee and brood area) may compatible with the monthly changes in correlations between hygienic and grooming behaviours. In March representing early spring period, June and July, lowest population rate were observed in the local habitat of Yığılca honey bee ecotype. In May, population growth rates reached the highest levels between April to May. Also nectar flow is emerged within this period and finished in July.

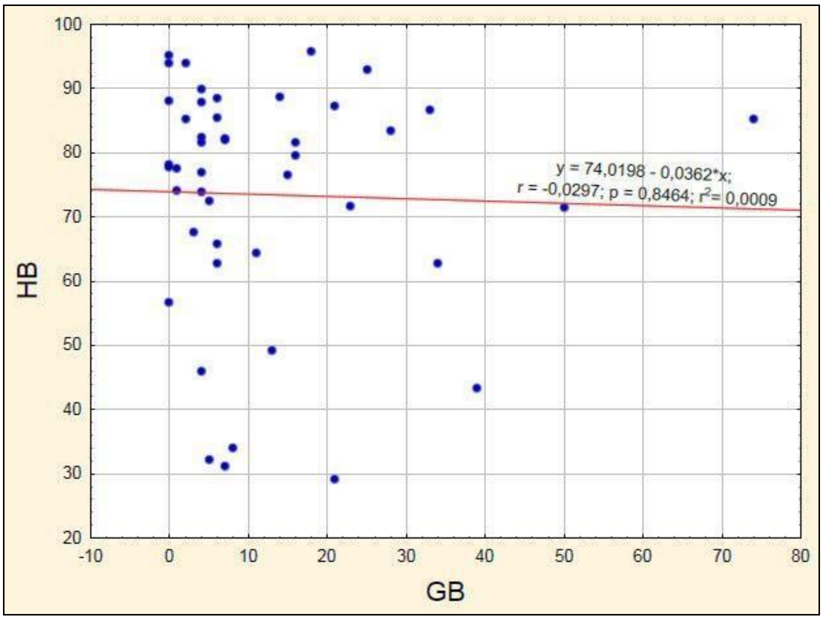

Fig 3: Negative relationships between HB-GB performances within March

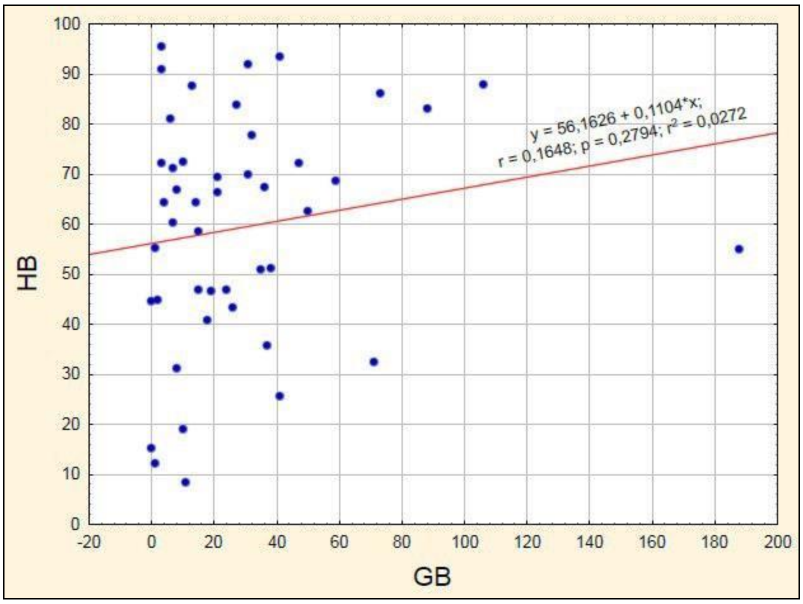

Fig 4: Positive relationships between HB-GB performances within April

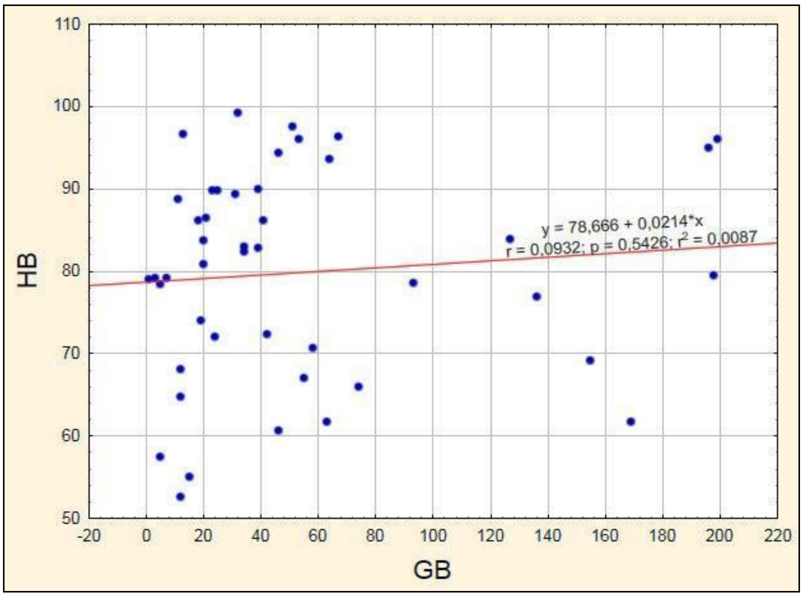

Fig 5: Positive relationships between HB-GB performances within May

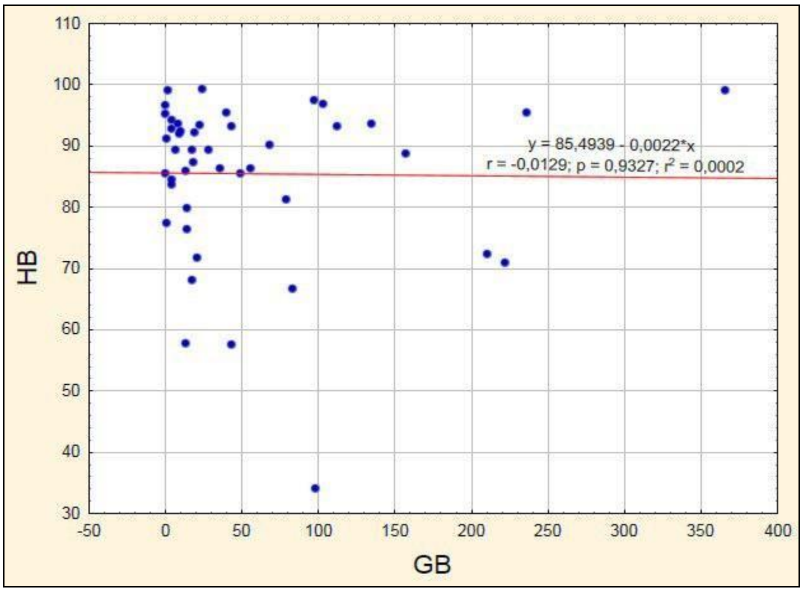

Fig 6: Negative relationships between HB-GB performances within June

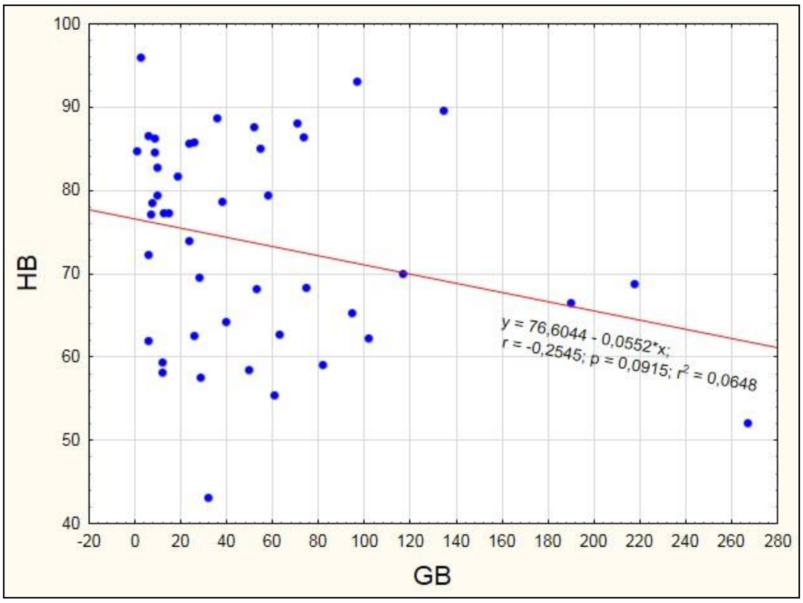

Fig 7: Negative relationships between HB-GB performances within July

Discussion

To increase colony strength and hygiene in honey bees, some behavioral mechanisms can emerge depending on genetic and environmental factors. Hygienic and grooming behaviours were activated under the effect of ectoparasitic infestations, mainly *Varroa destructor*, or other diseases (bacterial, viral, fungal etc.) (Boecking and Spivak, 1999, Anderson and Trueman, 2000) [8, 2]. HB shows high variations regarding to race (*A. m. intermissa*, *A. m. jemenitica*, *A. m. lamarckii*, *A. m. mellifera*) and also large terrestrial scale (Europea, Northern and Southern part of Africa, Arabian Penninsula) (Kamel *et al.* 2003, Jevtic *et al.* 2009; Balhareth *et al.*, 2012; Adljane and Haddad, 2014) [15, 14, 6]. Also, hygienic behaviour performance may change monthly within season (Adjlane and Haddad, 2014) [1]. For example, emphasized, populations of *Apis cerena* and *Apis mellifera* can show higher hygienic behaviour in autumn compared to spring (Lin et. al., 2016) [19]. Grooming behaviour (GB) is an activity that damages body parts of *V. destructor* (Guzman-Novoa *et al.*, 2012; Nganso *et al.*, 2017) [12, 22]. Le Conte *et al.* (2007) [18] suggested that local populations of *A. mellifera* not exposed to drug treatments showed stronger GB performance against *V. destructor*. Andino and Hunt (2011) [3] noted that the emerging decrease in the mite population under laboratory conditions was associated with the increase of the GB performances in the honey bees. However, Stanimirović *et al.* (2010) [29] stated that inheritance of the GB tendency was not seen over generations. Guzman-Novoa *et al.* 2012; Nganso *et al.* (2017) [12, 22] stated *V. destructor* infestation levels have a decisive influence on the emergence of GB. According to some studies, activities and also relations of HB and GB performances promote to colonial surviving and correlations. These performances could be convert positive or negative relationships under the effects of genetic and environmental conditions during the same season (Arechavaleta-Velasco and Guzman-Novoa 2001) [5, 12].

Our findings indicated that, bi-directional monthly changes between HB and GB in the same season may occur depending on the population development during the season for the Yığılca ecotype. Moreover, although negative correlations were determined in March, June and July, differences also can be observed between the frequency of behaviours in phenotype. *Varroa* infestation level is higher in broods during April and May. According to the data we obtained, in this direction, while HB frequency and effectiveness come into prominence against *Varroa* contamination to broods, GB activity remains at low levels at the beginning of the season. It is possible to see a negative correlation between the two behaviours in March. April and May are the most active months for the Yığılca ecotype regarding to the nectar flow. Therefore, *Varroa* infestation levels reaches high levels due to the increasing number of broods and also adult bee populations. With the increase in *Varroa* infestation on adult bees, the incidence of GB in the phenotype also increases, so that positive linear relationships can be observed between HB-GB during these two months. June and July represents the periods when the rate of brood area is lowest, and the density of adult bees is higher. For this reason, despite its effectiveness in HB, the increase in GB may come to the fore, and the correlations can turn into a negative direction after the nectar flow.

Conclusion

HB and GB, two genetically inherited traits, are directly responsible for colony healthiness and strengths unlike other performances. When the population and brood development change oppositely, the effectiveness of the behaviours may alter the relationships positively or negatively. This alteration may depend on the biological periods of honey bee social castes when the beginning of springs and after the main nectar flow. That can be explain why grooming and hygienic behaviours rates change depends on season.

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