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A Model for Determining the Interactions Between some Honey Bee (*Apis mellifera* L.) Genotypes and Different Grooming Times in Terms of Aggression

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ABSTRACT

In honey bee (*Apis mellifera* L.), aggression and aggressive behavior occur due to many reasons and the most important one is the genotype. However, regardless of genotype, a bee colony can have different levels of aggression at different times. If the bee's aggression is not due to racial characteristics, this may indicate some problems in the colony. One of the most important reasons is the absence of queen bees in the colony, and the other is that the amount of nutrients in the hive has decreased to a critical level. Some other environmental effects are effective on bees' aggression and aggressive behavior. In this study, the determination of the relationships between the aggression of four different honey bee genotypes (Carniolan, Caucasian, Black Sea and Muğla) in Samsun province in two different months (July, August) at different times of the day (09:00-13:00-17:00) was intended. For this purpose, a Log-linear model analysis was made. According to the findings, genotype, month and time, which are the main effects, and genotype × month interaction among the second-order interactions were found to be statistically significant, while genotype * hour and month × hour interactions were not significant. In Samsun province, it was determined that the Carniolan genotype was 7.846 times less, the Caucasian genotype 3.991 times and the Black Sea genotype 3.888 times less aggressive than the Muğla genotype. In addition, it was determined that the aggression in July was 1.185 times less than in August, and they were less aggressive in the daytime than in the evening and morning hours. It was determined that the Carniolan genotype was 2.04 times less aggressive than the Muğla genotype in July. Before and after the nectar flow seasons, studies to determine the aggression for all genotypes suitable for the regions will be a good source of information for beekeepers and researchers.

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Introduction

Honey bees (*Apis mellifera* L.) are insects that live in colonies with a complex social organization and order, as is the case with most social insects. Their nests contain food stores in the form of high-carbohydrate honey (Berenbaum and Calla, 2021), lipid and protein-rich pollen, as well as protein-rich bee larvae and colony individuals (queen, drones and worker bees). The honeybee is an attractive food source for a wide variety of predators and pests due to these rich food sources and colony individuals (Breed et al., 2004). These food sources and colony individuals should be defended against various predators, pests, parasites, worker bees that are not from their colonies, and even beekeepers interested in colony management affairs. To conserve these resources, honey bee, like some other social insects, have developed a defensive behavior that involves communication and division of labor among the members of the colony (Avalos et al., 2020). Worker bees from honeybee colony individuals perform most of the tasks such as nectar, pollen, collecting water and defense of the colony outside the colony, while performing jobs such as brood care, cleaning and honeycomb processing in the colony. The performance of these processes in the honey bee colony by the worker bees depends primarily on time, depending on genetic differences. Worker bees can perform different tasks depending on their age (Arechavaleta-Velasco et al., 2003; Robinson and Page, 1988; Seeley, 1985; Winston, 1987). Generally, young worker bees take care of the center of the nest and feeding the brood (larvae) with brood food, while middle-aged worker bees carry out operations such as honey and pollen storage at the edges of the nest. Older or field worker bees carry nectar, pollen and water to the colony (Moritz and Southwick, 1992; Seeley, 1985; Winston, 1987; Hunt et al., 2003). Worker bees of all age groups do not do the same job, even if the division of labor in the colony is made depending on age. Some worker bees specialize in performing only one or more of their tasks (Robinson and Page, 1988; Trumbo et al., 1997). Studies have shown that this specialization is partly due to genetic structure and genetic variability among individuals in the colony, which is a factor affecting the division of labor among worker bees in the colony (Breed et al., 1990; Frumhoff and Baker, 1988; Guzmán-Novoa et al., 2002; Page et al., 2000; Robinson and Page, 1988, Trumbo et al., 1997; Tugrul et al., 2000). Defensive behavior or aggression in honey bee colonies is also a complex system with significant heritability at the colony level, influenced by many interacting environmental and genetic factors (Anholt and Mackay, 2012), and its implementation, harshness, and duration vary depending on environmental influences (Avalos et al., 2017). Guard worker bees at the entrance to the colony exhibit defensive behaviors by interception predators, non-nestmate workers and other arthropods away from the colony and releasing alarm pheromones (Moore et al., 1987). Defense of the colony is a behavior that 10-15% of the worker bees in the colony exhibit and it is considered a special task (Hunt et al., 2003; Moore et al., 1987). However, worker bees in the colony react to the alarm pheromone and fly towards moving targets. These worker bees come out of the colony to pursue and sting a target (Breed et al., 2004). The distinction between worker bees that exhibit protective behavior and worker bees that react by sting is ambiguous. Researchers determined the number of worker bees exhibiting stinging behavior at the colony level in order to determine the defense level of the colonies. In front of the flight hole of the colony, aggression was detected by the number of needles left on it by shaking the ball made of leather or suede with a diameter of 4 cm for 1-2 minutes (Guzmán-Novoa et al., 2002).

The most important behavior of a honey bee colony is breeding and colony defense (Atkinson and Ellis, 2011; Uzunov et al., 2014). Although the defense behavior of honey bees is advantageous in terms of evolution and ecology, it is not advantageous for people who make use of honey and other products of honey bees (Guzman-Novoa et al., 2002) and especially for beekeepers. Defensive behavior often complicates colony management (Andere et al., 2002) and is added to breeding programs to use gentleman bees in breeding. However, it is also very important to defend the honey bee colony and its resources and to maintain colony integrity (Hunt et al., 2003).

Most of the studies on aggression have examined differences between races, time, or periods of care (Akyol et al., 2003; Yucel and Kosoglu 2011; Cengiz and Erdogan, 2018). In this study, it was aimed to determine the relationships between four different honey bee genotypes in two different months and different hours of the day for the aggression of honey bees.

Material and Method

Material

Honey bee colonies representing the Caucasian genotype and the Black Sea genotype, whose aggressive behaviors were determined and evaluated in the study, were selected from the colonies at Ondokuz Mayis University, Faculty of Agriculture, Beekeeping Research and Application Unit. Carniolan and Mugla genotypes were selected from colonies in two separate private enterprises working with these genotypes. In order to equalize the environmental conditions, attention was paid to the fact that the selected colonies had the same worker bee stock (7 bee frames/colony). The apiaries containing the colonies used in the experiment are located in an area of approximately 7 km radius. A 5 cm diameter suede cloth-covered ball was used to determine the aggressiveness of the colonies. In determining the aggression, the number of needles remaining on the ball was determined by swinging the ball for 1 minute so that the flight hole of the hive was not closed (Guzmán-Novoa et al., 2002). Ten colonies from each genotype were studied with a total of 40 colonies and the total number of needles was evaluated. SPSS 21 packages licensed by Ondokuz Mayis University was used in the analysis of data.

Method

Cross tables are used to determine the relationships between variables in the analysis of classification and sorting type data that have qualitative characteristics. While the relationships in two-way cross tables can be determined by chi-square analysis, log linear models (LLM) are used in determining the relationships in three or more (k) directional tables and they are also named as “Multiway Frequency Analysis” (Topaloglu and Atay, 2020). Log- Linear models are a special case of the general linear model that includes regression and ANOVA models that examine the binary interactions between variables as well as three and more directional interactions. The main purpose of this method is to find the most suitable model by comparing the saturated model predicting cell frequencies with reduced models (Garson, 2012). This method is usually explained in 5 steps. In the first step, the model is suggested. In the second step, the expected frequencies are calculated with the assumption that the model is suitable. In the third step, the observed frequencies and the expected frequencies are compared. In the fourth step, the rejection or acceptance of the model is decided. In the fifth and last step, if the model is accepted, the results are interpreted, if rejected, another model is tried (Burnett, 1983; Simsek Kandemir and Simsek, 2019). If the parameters included in the model include all the main effects and all interactions between the main effects, the saturated model is called the unsaturated model if it is created by subtracting some of the negligible interaction effects (Agresti, 2002; Koleoglu, 2018). The saturated model created in this study to determine the relationships between 4 different bee genotypes (G), two different months (M) and different times of the day (T) for the aggressive behavior is given below.

$$\ln f_{ijk}=\theta+\lambda_i^G+\lambda_j^M+\lambda_k^T+\lambda_{ij}^{GM}+\lambda_{ik}^{GT}+\lambda_{jk}^{MT}+\lambda_{ijk}^{GMT}$$

where θ is the constant parameter; λ_i^G,λ_j^M,λ_k^T are the main effects of i. level of honey bee genotype, j. level of month, k. level of time, respectively. λ_{ij}^{GM}, λ_{ik}^{GT}, λ_{jk}^{MT}, refers to double and λ_{ijk}^{GMT} triple interactions.

In the LLM, the standardized estimate of each λ in the saturated model is first calculated before the reduced model is calculated. Once the most important effects are identified, as few effects as possible are included in the model. The fit of the last reduced model is determined by the chi-square test (χ^2) and the likelihood-ratio statistics (G^2) tests. H_0 (the model is significant) and H_1 (the model is insignificant) hypotheses are tested. If the goodness of fit test is statistically insignificant, the model is used (P>0.05). Otherwise, additional effects are added to the model until a suitable model is found (Hintze, 2007).

$$\chi^2=2\sum_{i,j,k}\frac{(f_{ijk}-\widehat{m}_{ijk})^2}{\widehat{m}_{ijk}} \qquad G^2=2\sum_{i,j,k}f_{ijk}\ln\left(\frac{f_{ijk}}{\widehat{m}_{ijk}}\right)$$

where f_{ijk}: cell frequencies, $m_{ijk}=E(f_{ijk})$: expected cell frequency. When the m_{ijk} is estimated using maximum likelihood, the results are displayed $\widehat{m}_{ijk}$.

In this study, the most appropriate model to determine the interactions between the aggressive behaviors of 4 different honey bee breeds (G) at two different months (M) and at different times of the day (T) was determined as follows.

$$\ln f_{ijk}=\theta+\lambda_i^G+\lambda_j^M+\lambda_k^T+\lambda_{ij}^{GM}$$

where θ is the constant parameter; $\lambda_i^G,\lambda_j^M,\lambda_k^T$ are the main effects of i. level of honey bee genotype, j. level of month, k. level of time, respectively. λ_{ij}^{GM} is genotype*time interactions effects.

According to the results of the likelihood ratio test and Pearson's chi-square test, it was determined that the p-value of the obtained model was greater than 0.05 (H_0: the model is significant) statistically not significant, that is, the hypothesis H_0 cannot be rejected, but the model is significant and this model can be used for the prediction (Table 1).

In the interpretation of the model coefficients, odds ratio values, which are detailed in the studies of Bagdatli Kalkan (2018) and Topaloglu and Atay (2020), were used.

Results and Discussion

The summary findings of the effects and interactions for the model created to determine the relationships of 4 different honey bee genotypes in two different months and at different hours of the day for the aggressive behavior examined in the study are given in Table 2.

When the first line of the findings (K = 1) of K-way and higher order effects in Table 2 is examined, it can be stated that the main effects ($\lambda_i^G,\lambda_j^M,\lambda_k^T$), double interaction effects ($\lambda_{ij}^{GM},\lambda_{ik}^{GT},\lambda_{jk}^{MT}$) and triple interaction effects (λ_{ijk}^{GMT}) are significant according to the likelihood ratio and Pearson chi-square test results (p<0.05). In the second line (K=2), it can be stated that second ($\lambda_{ij}^{GM},\lambda_{ik}^{GT},\lambda_{jk}^{MT}$) and third-order effects (λ_{ijk}^{GMT}) are significant according to the likelihood ratio and Pearson chi-square test results. However, in the third line (K=3), the third-order interaction effect (λ_{ijk}^{GMT}) was not statistically significant (P>0.05). When K-way interactions were examined, it was determined that the main effects and second-order interaction effects were significant (P<0.05), while the third-order interaction effects were insignificant (P>0.05) according to the likelihood ratio and Pearson chi-square test results. According to these results, it was determined that the most suitable model for the data would be in accordance with the unsaturated hierarchical linear model that includes the parameters of main effects and second-order interaction effects but not third-order interaction effects. Partial chi-square and probability values of effects and interactions are given in Table 3 in order to determine the second-order interaction parameters in the model examined.

Table 1. Goodness of fit criteria for model

Tests	Values	df	P-values
Likelihood ratio	9.702	14	0.784^{NS}
Pearson's chi-square	9.749	14	0.780^{NS}

df: degree of freedom; NS: Non significant

Table 2. Three-way effects summary table

	K	df	Likelihood ratio		Pearson	
			Chi-square	P-values	Chi-square	P-values
K-way and higher order effects	1	23	1341.26	<0.001***	1706.91	<0.001***
	2	17	36.59	0.004**	35.95	0.005**
	3	6	3.54	0.739^{NS}	3.57	0.735^{NS}
K-way effects	1	6	1304.66	<0.001***	1670.97	<0.001***
	2	11	33.05	0.001**	32.38	0.001**
	3	6	3.54	0.739^{NS}	3.57	0.735^{NS}

df: degree of freedom; NS: Non significant; **significant (P<0.01) ***significant (P<0.001)

Table 3. Partial chi-square and probability values for effects and interactions

Effects	df	Partial Chi-square	P
Genotype*Month	3	26.64	<0.001***
Genotype *Time	6	1.28	0.973^{NS}
Month*Time	2	4.63	0.099^{NS}
Genotype	3	1007.26	<0.001***
Month	1	42.69	<0.001***
Time	2	254.71	<0.001***

df: degree of freedom; NS: Non significant; ***significant (P<0.001)

Table 4. Parameter estimates of effects and interactions

Effects	Parameters	Estimate	Std Error	Z	Odds ratio §	P-values	95% Confidence Interval	
							Lower Bound	Upper Bound
	Constant	5.448	0.054	100.146	232.29	<0.001***	5.342	5.555
Genotype	Carniolan (1)	-2.060	0.139	-14.774	7.846	<0.001***	-2.333	-1.787
	Caucasian (2)	-1.384	0.105	-13.215	3.991	<0.001***	-1.589	-1.179
	Black Sea (3)	-1.358	0.104	-13.102	3.888	<0.001***	-1.561	-1.155
	Mugla (4) (R)	0	.	.	-	.	.	.
Month	July (1)	-0.170	0.069	-2.448	1.185	0.014*	-0.305	-.034
	August (2) (R)	0	.	.	-	.	.	.
Time	9.00 (1)	-0.420	0.063	-6.695	1.522	<0.001***	-0.542	-.297
	13.00 (2)	-1.202	0.082	-14.641	3.327	<0.001***	-1.363	-1.041
	17.00 (3) (R)	0	.	.	-	.	.	.
Genotype×Month	11	-0.713	0.252	-2.824	2.040	0.005**	-1.207	-0.218
	12	0	.	.	-	.	.	.
	21	-0.716	0.187	-3.838	2.046	<0.001***	-1.082	-0.351
	22	0	.	.	-	.	.	.
	31	-0.532	0.175	-3.042	1.702	0.002**	-0.875	-0.189
	32	0	.	.	-	.	.	.
	41 (R)	0	.	.	-	.	.	.
	42	0	.	.	-	.	.	.

*significant (P<0.05); **significant (P<0.01); ***significant (P<0.001); (R): Reference; §: For only negative (-) estimates, odds ratios were found by dividing negative estimates coefficients ($1/e^{estimate}$) and the interpretations were reversed. For example, rather than more aggression, less aggression.

When Table 3 is examined, genotype, month, hour, which are the main effects affecting the aggression in honey bees, genotype×month interaction among the second-order interactions were found to be statistically significant (P<0.05), genotype*time and month*time interactions were found to be statistically insignificant (P>0.05). Findings of the parameter estimations of main effects and interactions are given in Table 4. In Table 4, unnecessary parameters are shown with zero (0).

When Table 4 is examined, it was determined that the aggression of the Carniolan (1), Caucasian (2) and Black Sea (3) genotypes in the model was statistically significant (P<0.05). It was determined that Carniolan genotype was 7.846 ($1/e^{-2,060}$) times less, the Caucasian genotype 3.991 ($1/e^{-1,384}$) times and the Black Sea genotype 3.888 ($1/e^{-1,358}$) times less aggressive than the Mugla genotype.

When the aggression levels of honey bee colonies evaluated in the experiment were examined by months, it was determined that the aggression in July was 1.185 ($1/e^{-0.170}$) less compared to August, that is, bees were more aggressive in August compared to July.

When the bees' aggression was examined according to the time of the day, it was determined that they were 1.522 ($1/e^{-0,420}$) times less aggressive at 9:00 than at 17:00, while at 13:00 they were 3.327 ($1/e^{-1,202}$) times less aggressive than at 17:00, and these coefficients were statistically significant (P<0.05).

When the aggression of honey bee genotypes was examined by months, it was determined that the aggression of the Carniolan genotype in July was 2.040 times ($1/e^{-0,713}$) less than the Mugla genotype in July, the Caucasian genotype was 2.046 times ($1/e^{-0,716}$) less and the Black Sea genotype was 1.702 times ($1/e^{-0,532}$) less. Also, it was determined that these coefficients were statistically significant (P<0.05).

Akyol et al. (2003) investigated the aggression behavior of Caucasian (♀) x Caucasian (♂), Mugla (♀) x Mugla (♂), Caucasian (♀) x Mugla (♂) and Mugla (♀) x Caucasian (♂) genotype groups in different periods. In the analysis of variance applied to determine the difference between genotypic groups in terms of aggression, the differences between genotypes and periods and genotype x period interaction were found to be significant (P<0.01). In their study, they found that genotypes were different and that colonies with Mugla queen bees (MxM and MxC) were more aggressive than Caucasian queen bees (C×C and C×M) at all measurement periods. These results obtained support the results of our study.

Cengiz and Erdogan (2018) determined the aggression of Caucasian, Carniolan, Buckfast and Erzurum genotypes in their study. They found significant differences between genotypes, and it was determined that Caucasian and Carniolan genotypes were similar to each other and were less aggressive than Buckfast and Erzurum genotypes, and Erzurum genotype was the most aggressive.

Yucel and Kosoglu (2011) examined some performance characteristics and aggressiveness of Mugla ecotype and Italian hybrid honey bees in Aegean Region conditions and determined that Mugla bees were more aggressive than Italian bees in their aggression tendency measurements.

Akyol et al. (2003) and Yucel and Kösoglu (2011) reported that in the genotypes they examined, aggression was less during the nectar flow period. In our study, it was determined that bees were more aggressive in August compared to July. The fact that the nectar flow in the region where we conducted the experiment is higher in July supports that the colonies are calmer during the nectar flow period. While examining the differences between genotypes in terms of aggression behaviors in other studies, the status of the races considered in this study was determined according to the most combative race and month.

Conclusion

Honey bee breeders generally complain about the aggression of bees and generally want to work with colonies with a calmer temperament. For some bee genotypes, non-stinging bee expression is used. In the same apiary, some colonies behave very calmly and do not show stinging behavior towards the beekeeper during colony care, while some bees are extremely aggressive. Regardless of the genotype, a bee colony can have different levels of aggression at different times. If the bee's aggression is not due to genotype characteristics, this may indicate some problems in the colony. One of the most important causes of aggressive behavior and aggression in bees is the absence of queen bees in the colony. Another important problem is that the amount of nutrients in the hive is at a critical level. During periods when nectar resources are abundant in the field, bees are generally very calm. Some other environmental effects are effective on bees' aggression. Knowing the aggression levels of bee genotypes by beekeepers and knowing the months and hours of the day when they are particularly aggressive can prevent bee losses due to sting and will contribute to beekeepers doing their job more comfortably. According to the results obtained from this study, it was determined that the Carniolan race in Samsun province is calmer than Caucasian, Black Sea and Mugla genotypes. In addition, it was determined that the bee genotypes examined were calmer in July compared to August and that they were less aggressive during the day than in the evening and morning hours. It has been determined that the genotypes are in interaction with the months. Also, in terms of aggression, it was determined that genotypes have an interaction effect with months. Based on the results of this study, conducting studies to determine aggression for all genotypes raised in the regions, especially before and after the nectar flow seasons of the regions, will create a good source of information for beekeepers and researchers.

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