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COMPARISON OF PURE CAUCASIAN, CARNIOLAN AND NATIVE ANATOLIAN ECOTYPE HONEY BEE (*Apis mellifera* L.) COLONIES IN THE EASTERN ANATOLIA REGION WITH RECIPROCAL F1 HYBRIDS

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Abstract

The aim of this study was to compare specific physiological and behavioral characteristics of *A. m. caucasica*, *A. m. carnica*, and native honeybees, as well as reciprocal crosses, within the ecological conditions of East Anatolia (Van province), Turkey. The findings revealed that native honeybees exhibited the highest average sealed brood area and the highest average number of frames with bees. In contrast, the highest average honey yield was observed in the crossbreeding of Carniolan bees with native bees. Significant differences were identified among the groups concerning brood area, the number of bee frames, aggression tendency, and honey yield. The study concluded that Carniolan and Caucasian bees exhibited suboptimal performance outside their native habitats. Nevertheless, Carniolan bees were identified as a viable option for mitigating aggression tendencies and enhancing honey yield in native bee populations.

Keywords: *Apis mellifera caucasica. Apis mellifera carnica. Apis mellifera anatoliaca*. Colony Performance. F_1 Crosses.

1. Introduction

Ilyasov et al. (2020) reported that various honeybee subspecies, which originated in diverse climatic conditions, are currently widespread across the globe, as referenced by several researchers. Anatolia, acknowledged as a primary habitat for honeybees, along with Asia and Africa (Ruttner 1988; Kambur and Kekeçoglu 2018; Özkırım 2018; Acar and Kekeçoglu 2020; Ünalan and Akyol 2021), hosts numerous honeybee breeds and ecotypes, distinguished by morphological, physiological, and behavioral characteristics (Bouga et al. 2011; Arslan 2020; Kükrer et al. 2021; Ünalan and Akyol 2021).

In earlier research, Caucasian bees (*A. mellifera caucasica*) were documented as prevalent in the north-eastern regions of Turkey, whereas Iranian bees (*A. mellifera meda*) and Syrian bees (*A. mellifera syriaca*) were reported in the south-eastern parts of the country. Carniolan bees (*A. mellifera carnica*) were found in Thrace, and Anatolian bees (*A. mellifera anatoliaca*) were identified in other regions (Ruttner 1988; Cengiz and Erdoğan 2017; Arslan 2020; Kekeçoğlu et al. 2021). However, these findings have been subject to discussion in Turkey due to unregulated queen bee sales and migratory beekeeping activities, as indicated by Frunzeun et al. (2021).

It is inherent that honeybee breeds exhibit elevated survival and yield rates within their natural ranges. Conversely, uncontrolled hybridizations contribute to the degeneration of genotypes adapted to

specific regions, resulting in diminished yields. Therefore, comparing the performances of genotypes under controlled breeding conditions could contribute to enhance productivity. In this study, the performance and viability of pure and crossbred hybrids of Caucasian and Carniolan bees, along with their genotypes, which have long been bred in the region, were systematically analyzed under local conditions.

2. Material and Methods

Material

The research was conducted in the province of Van, situated between longitudes 42° 40' and 44° 30' east, and latitudes 37° 43' and 39° 26' north. Van, with a central altitude of 1725 m, is located within the closed basin of Lake Van in the Upper Murat-Van Section of the Eastern Anatolia Region in Turkey. The climate is continental, characterized by severe and prolonged winters. Summers experience high temperatures and dry conditions, with approximately 20 days above 30°C. Annual precipitation ranges from 370 mm to 570 mm. The area consists largely of a high plateau with a steppe structure (Karaca et al., 2019). The study was conducted at the conclusion of a drier summer following a harsh winter, aligning with the typical climatic characteristics of the province. In summary, it was not a productive season for beekeeping.

The study utilized *A. m. caucasica*, *A. m. carnica*, and native honeybees. The *A. m. caucasica* bees were obtained from the Ardahan Caucasian Bee Production, Training, and Gene Center Directorate, whereas the *A. m. carnica* bees were procured from a commercial corporation. The native honeybees were sourced from a breeder located in an area distant from any migratory beekeeping activities over an extended period of time.

In order to produce pure and reciprocal hybrids of the three genotypes, queen bees from breeding colonies underwent insemination by males of the appropriate genotype. The resulting groups included CarniolanXCarniolan ($CR^{♀}XCR^{♂}$), CarniolanXCaucasian ($CR^{♀}XC^{♂}$), CarniolanXNative ($CR^{♀}XN^{♂}$), CaucasianXCaucasian ($C^{♀}XC^{♂}$), CaucasianXCarniolan ($C^{♀}XCR^{♂}$), CaucasianXNative ($C^{♀}XN^{♂}$), NativeXNative ($N^{♀}XN^{♂}$), NativeXCarniolan ($N^{♀}XCR^{♂}$), and NativeXCaucasian ($N^{♀}XC^{♂}$). The queen bees, upon initiating egg-laying in the nucleus hives, were introduced into hives that had been equalized based on the number of frames with brood and bees (6 frames with bees and 2 frames with brood).

Methods

In this study, assessments of sealed brood areas and number of frames covered with adult bees were conducted 6 times at 21-day intervals. The research groups were established in September 2010, with the initial data for colony performance (brood area and number of bee frames) collected on September 2. Following the formation of largely similar colonies, they underwent overwintering. Subsequent measurements were taken on May 6, May 27, June 17, July 8, and July 29 of 2011, to track colony development. Honey harvest was performed on August 17. The sealed brood areas of colonies were determined using the Puchta method and expressed in square centimeters (Tunç et al. 2020).

To assess the level of colony aggression, black tennis balls with a diameter of 5 cm were employed. This assessment was conducted three times on June 18, July 9, and July 21. The aggression tendency was determined by counting and recording the bee stings on the balls, which were suspended in front of the bee flight holes for a duration of 1 minute.

Honey yield was recorded post-harvest using the standard measure of 2 kg of honey per frame with bees.

Statistical analysis

Repeated Measures Analysis of Variance (ANOVA) was employed to assess the differences across the periods, considering that the sealed brood area measurements were treated as repeated measures and exhibited a normal distribution.

Periodic measurements for the same study groups were analyzed using SAS Software, and the characteristic differences among the groups were determined through the Duncan test [SAS, 2020]. The study initially began with a total of 61 colonies but concluded with 48 colonies due to colony losses and swarming.

3. Results

Brood area

The brood areas observed for the study groups are presented in Table 1 and Figure 1. The analysis of variance conducted on the data revealed that the differences among groups were statistically significant ($P < 0.01$), whereas the GroupXPeriod Interaction was found to be not significant.

The findings from the Duncan Multiple Range Test indicated that the N♀XN♂ and CR♀XN♂ groups were significantly different from the N♀XCR♂, C♀XC♂, CR♀XCR♂, and CR♀XC♂ groups.

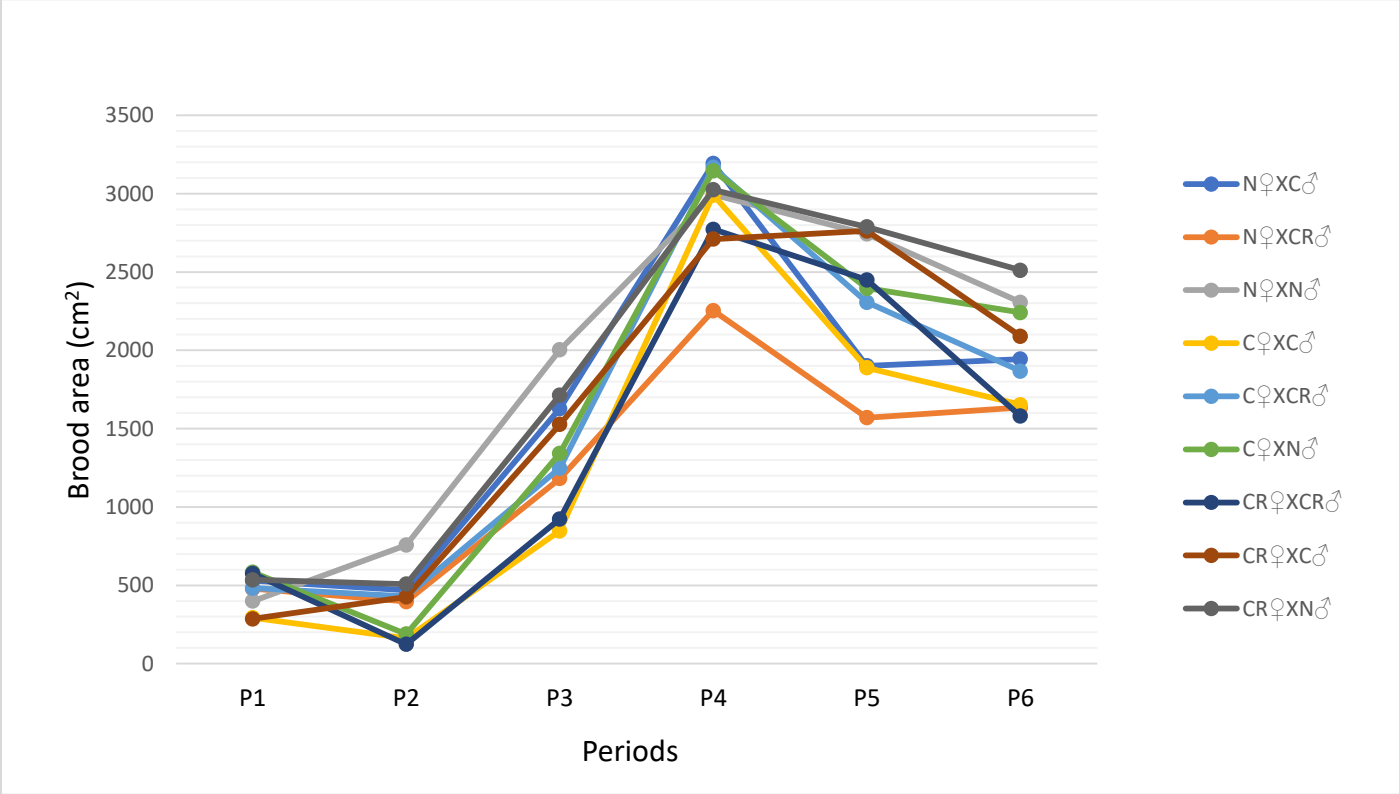

Figure 1. Sealed brood area according to genotype and time period.

It was observed that the genotype groups exhibited distinguishable brooding activity in the Van Province, where the study was conducted. Analysis of the brooding rates during the test revealed that the native group (N♀XN♂) exhibited a higher mean brooding rate compared to the other groups. Additionally, the CR♀XN♂ group also demonstrated a noteworthy brooding rate. Conversely, the genotypes with the lowest brood areas were C♀XC♂ and N♀XCR♂. Other notable findings included the elevated brooding rate in the native honeybee group under regional conditions and the observation that pure Caucasian and Carniolan genotypes generally exhibited lower mean brooding rates.

An important prerequisite of the Repeated Measures Analysis is to assess the equality of variances among measurement levels, and the Mauchly test serves this purpose. According to the results of this test, if the significance level obtained is greater than 5%, it is accepted that the variances among measurements are equal, and the assumption of sphericity is considered valid. In this study, the Repeated Measures Analysis was conducted to analyze the change in sealed brood area over the test periods, and Mauchly's test was employed to evaluate sphericity, which is the fundamental assumption of this analysis (see Table 2).

Table 1. Sealed brood area results (cm^2) according to genotype and time period.

Genotype	Periods												General	
	1		2		3		4		5		6			
	n	Brood area	n	Brood area	n	Brood area	n	Brood area	n	Brood area	n	Brood area	n	Brood area
$N^{♀}XC^{♂}$	8	529.4±122.4	7	469.3±182.5	7	1628.2±100.1	7	3192.4±454.2	6	1901.2±296.4	6	1943.9±216.2	41	1569.1±178.8^{BCDE}
$N^{♀}XCR^{♂}$	6	478.2±109.7	5	396.7±174.3	5	1181.8±256.0	5	2253.2±565.6	5	1570.3±307.1	5	1634.8±145.6	31	1227.5±162.3^{CDE}
$N^{♀}XN^{♂}$	8	399.4±42.0	8	756.6±115.7	8	2002.8±280.7	8	2994.3±237.1	7	2743.0±271.2	7	2306.1±114.4	46	1838.4±164.8^{A}
$C^{♀}XC^{♂}$	7	293.6±43.8	5	162.3±48.4	5	848.2±117.2	5	2991.0±249.1	5	1889.2±308.7	5	1652.5±193.6	32	1242.9±189.0^{E}
$C^{♀}XCR^{♂}$	6	482.5±57.8	5	430.5±229.0	5	1248.4±150.5	5	3167.1±436.6	5	2306.3±238.6	5	1867.5±150.0	31	1548.2±199.5^{ABCD}
$C^{♀}XN^{♂}$	5	582.9±67.8	5	188.6±31.8	5	1342.6±124.1	5	3145.61±287.0	5	2395.9±279.9	5	2241.6±259.6	30	1649.5±207.7^{ABC}
$CR^{♀}XCR^{♂}$	6	574.8±129.8	4	124.2±41.3	4	923.6±102.6	4	2772.4±624.0	4	2449.2±426.0	4	1580.4±150.5	26	1340.3±218.8^{DE}
$CR^{♀}XC^{♂}$	6	286.4±96.4	4	425.7±137.3	4	1527.4±112.0	4	2710.0±368.7	4	2761.9±132.2	4	2090.2±190.0	26	1530.0±214.8^{BCDE}
$CR^{♀}XN^{♂}$	9	535.0±90.7	8	507.4±142.8	8	1714.5±156.1	8	3024.4±362.2	7	2787.2±452.9	7	2510.5±264.7	47	1784.5±182.7^{AB}

Different letters indicate significant differences (p<0.01).

Table 2. Sphericity test results for sealed brood area.

Mauchly's Test of Sphericity							
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Periods	0.102	84.720	14	0.000	0.555	0.724	0.200

However, based on the results of Mauchly's sphericity test, it was determined that the assumption of sphericity was not valid ($p < 0.05$). The results of the comparison among periods for sealed brood area are presented in Table 3.

Table 3. comparison among time periods for sealed brood area.

Periods	Mean± Std. Error	p
1	500.846±36.290^{A}	<0.001
2	404.379±52.734^{A}	
3	1432.739±81.695^{B}	
4	2929.211±134.049^{C}	
5	2328.544±118.481^{D}	
6	2021.724±76.793^{E}	

Different letters indicate significant differences (p<0.001).

F4or the data in Table 3 for which sphericity was not assured, the Greenhouse-Geisser correction—one of the degrees-of-freedom correction methods applied in repeated-measures ANOVA—was less than 0.75. According to the Greenhouse-Geisser results, the difference among periods for sealed brood area was statistically significant ($p < 0.001$).

The change in sealed brood area over the study periods is depicted in Figure 2. The sealed brood area exhibited an increasing trend up to period 4, followed by a decreasing trend. This observed change was directly associated with honey bees' access to pollen and nectar sources.

Number of frames with bees

The number of frames with bees in the colonies was also assessed during the brood area measurements. The results for the number of frames with bees according to genotype and time period are provided in Table 4 and Figure 3. The ANOVA findings revealed that the differences among periods and groups were significant ($P < 0.01$), whereas the GroupXPeriod Interaction was found to be not significant.

Application of the Duncan Multiple Range Test on the data on number of frames revealed that the $N^{♀}XN^{♂}$ study group was similar only to the $CR^{♀}XN^{♂}$ group, and different from all the other groups. The data

also showed that the 5th and 6th periods were statistically similar, and that the 1st, 2nd, 3rd, and 4th periods were significantly different from each other (P < 0.05).

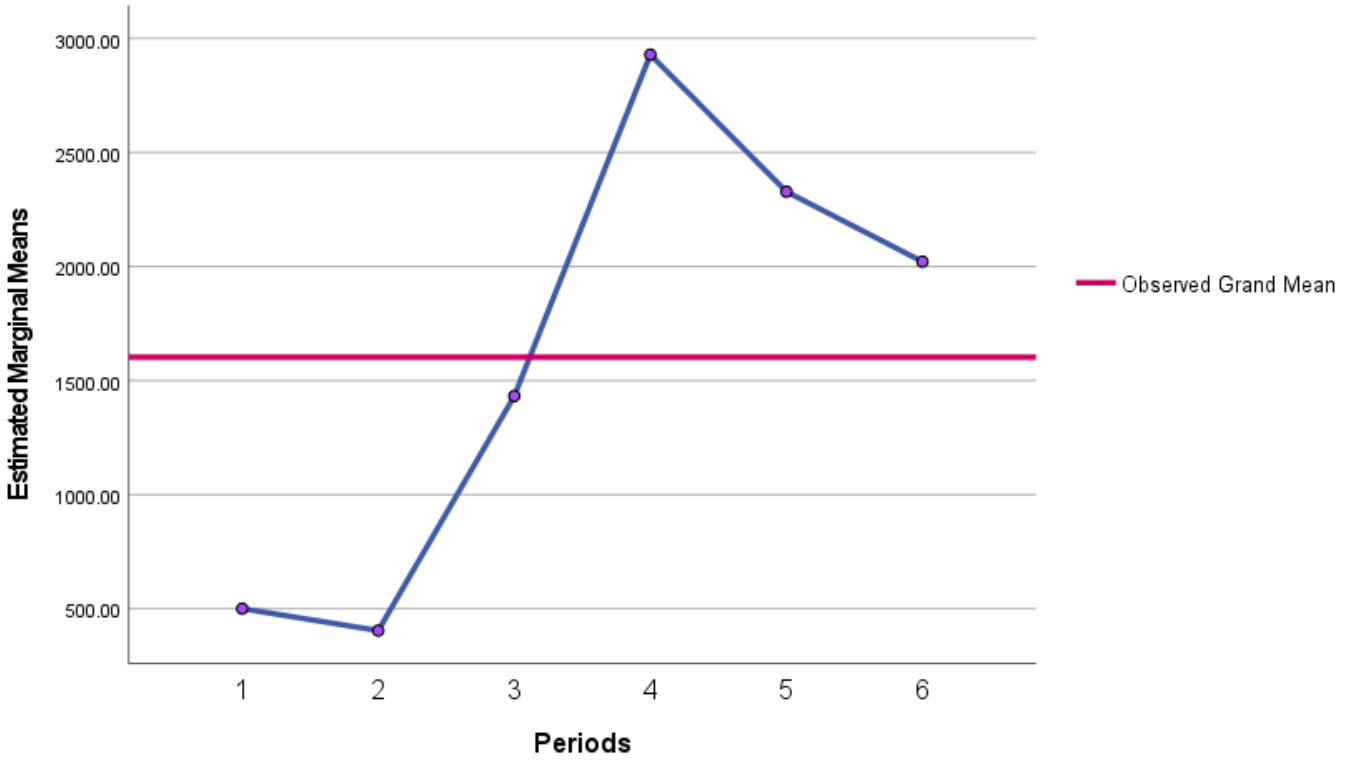

Figure 2. Change in sealed brood area over the study time periods.

Table 4. Number of frames covered with adult honey bees according to genotype and time period.

Genotype	Periods												General	
	1		2		3		4		5		6			
	n	Frame number	n	Frame number	n	Frame number	n	Frame number	n	Frame number	n	Frame number	n	Frame number
N♀XC♂	8	6.00±0.00	7	2.57±0.43	7	5.14±0.26	7	7.29±0.52	6	9.00±1.03	6	9.17±1.17	41	6.39±0.43^{BC}
N♀XCR♂	6	6.00±0.00	5	3.60±0.60	5	5.00±0.55	5	6.20±1.02	5	7.60±0.68	5	7.20±0.58	31	5.94±0.33^{C}
N♀XN♂	8	6.00±0.00	8	3.25±0.53	8	5.75±0.57	8	9.00±1.05	7	11.00±1.20	7	12.86±1.63	46	7.80±0.60^{A}
C♀XC♂	7	6.00±0.00	5	2.20±0.20	5	3.60±0.25	5	5.60±0.60	5	6.40±0.25	5	6.20±0.37	32	5.06±0.30^{D}
C♀XCR♂	6	6.00±0.00	5	2.40±0.40	5	4.60±0.00	5	7.20±0.97	5	8.80±0.37	5	8.00±0.55	31	6.16±0.44^{C}
C♀XN♂	5	6.00±0.00	5	2.20±0.20	5	4.40±0.25	5	6.60±0.40	5	8.20±0.80	5	8.60±1.08	30	6.00±0.46^{C}
CR♀XCR♂	6	6.00±0.00	4	1.50±0.29	4	3.75±0.48	4	6.75±0.95	4	7.75±0.95	4	7.25±1.11	26	5.77±0.55^{CD}
CR♀XC♂	6	6.00±0.00	4	2.75±0.25	4	5.00±0.4	4	7.75±0.85	4	8.25±0.63	4	9.00±0.58	26	6.42±0.45^{BC}
CR♀XN♂	9	6.00±0.00	8	2.63±0.88	8	5.75±0.53	8	8.75±1.01	7	9.86±1.24	7	11.14±1.16	47	7.19±0.52^{AB}
General	61	6.00±0.00^{C}	51	2.63±0.15^{E}	51	4.92±0.18^{D}	51	7.43±0.32^{B}	48	8.73±0.35^{A}	48	9.25±0.46^{A}		

Different letters indicate significant differences (p<0.01).

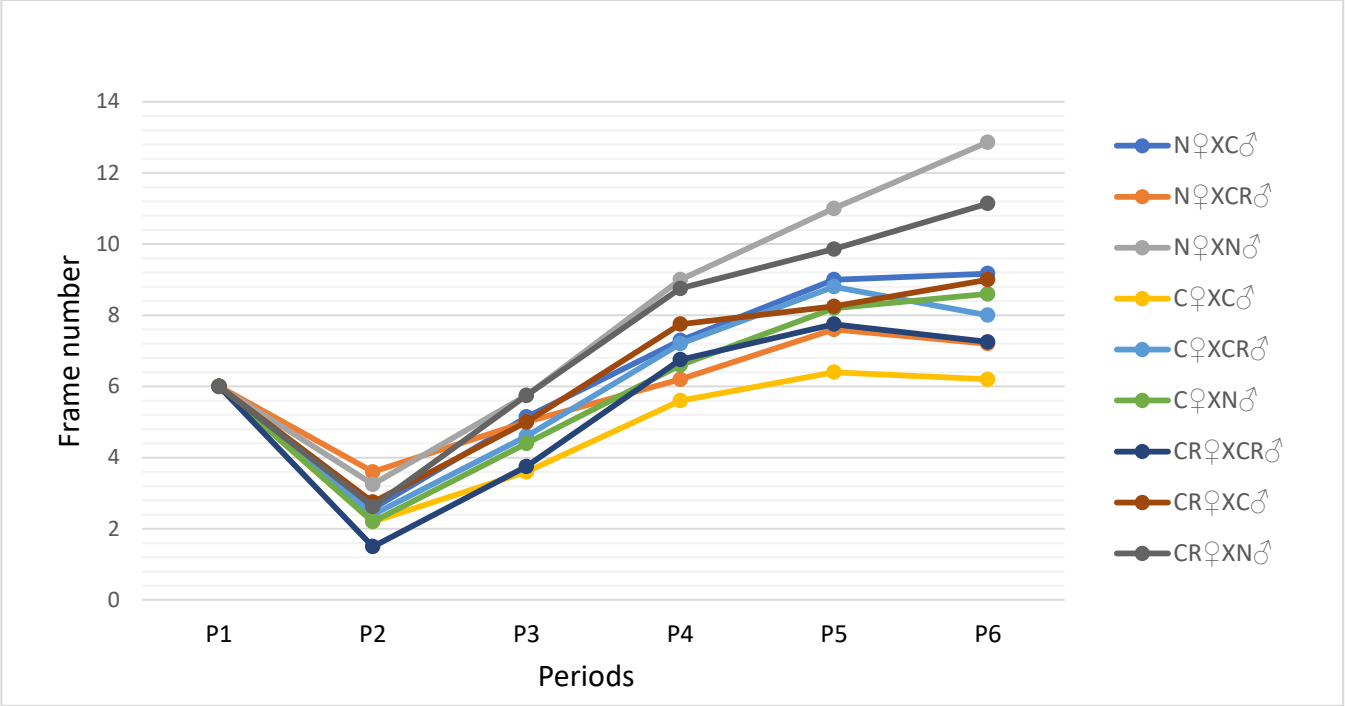

Figure 3. Number of frames covered with adult honey bees according to genotype and time period.

Defensive behavior

The ANOVA conducted on the data collected on the number of stings on the black tennis balls revealed that the difference among the time periods was not significant, whereas the differences among the study groups were significant ($P < 0.01$). The GroupXPeriod Interaction was also found to be not significant.

The number of stings according to genotype and time period are presented in Table 5.

Table 5. Number of stings (defensive behavior) according to genotype and time period.

Genotype	Periods						General	
	1		2		3			
	n	Sting number	n	Sting number	n	Sting number	n	$\bar{X} \pm S_{\bar{x}}$
N♀XC♂	4	7.50±1.66	3	10.00±1.53	3	9.33±1.20	10	$8.80±0.87^{B}$
N♀XCR♂	3	7.33±1.45	3	5.67±0.88	3	5.67±0.88	9	$6.22±0.62^{BC}$
N♀XN♂	4	10.75±2.25	3	23.00±5.29	3	18.00±1.53	10	$16.60±2.38^{A}$
C♀XC♂	3	4.33±0.88	3	2.33±0.88	3	4.67±1.20	9	$3.78±0.62^{CD}$
C♀XCR♂	3	1.67±0.88	4	3.50±1.26	4	5.25±0.63	11	$4.00±0.63^{D}$
C♀XN♂	3	5.00±0.58	4	4.25±1.11	4	6.00±0.91	11	$5.09±0.55^{CD}$
CR♀XCR♂	3	3.00±0.58	3	4.33±0.88	3	2.67±0.88	9	$3.33±0.47^{D}$
CR♀XC♂	3	3.33±0.88	3	4.33±0.88	3	4.67±1.88	9	$4.11±1.73^{CD}$
CR♀XN♂	4	4.75±1.11	3	6.67±2.60	3	9.00±2.65	10	$6.60±1.19^{CD}$
General	30	5.30±0.54	29	7.12±0.55	29	6.97±0.56		

Different letters indicate significant differences ($p<0.01$).

The results of the Duncan Multiple Range Test indicated that the behavior of the N♀XN♂ group was distinguishable, whereas the C♀XC♂, C♀XCR♂, C♀XN♂, CR♀XCR♂, CR♀XC♂, and CR♀XN♂ genotypes displayed similar aggression levels ($P < 0.05$). The most aggressive group was N♀XN♂, whereas the least aggressive was CR♀XCR♂. Figure 4 illustrates the defensive behavior of the study groups in terms of their number of stings according to time period.

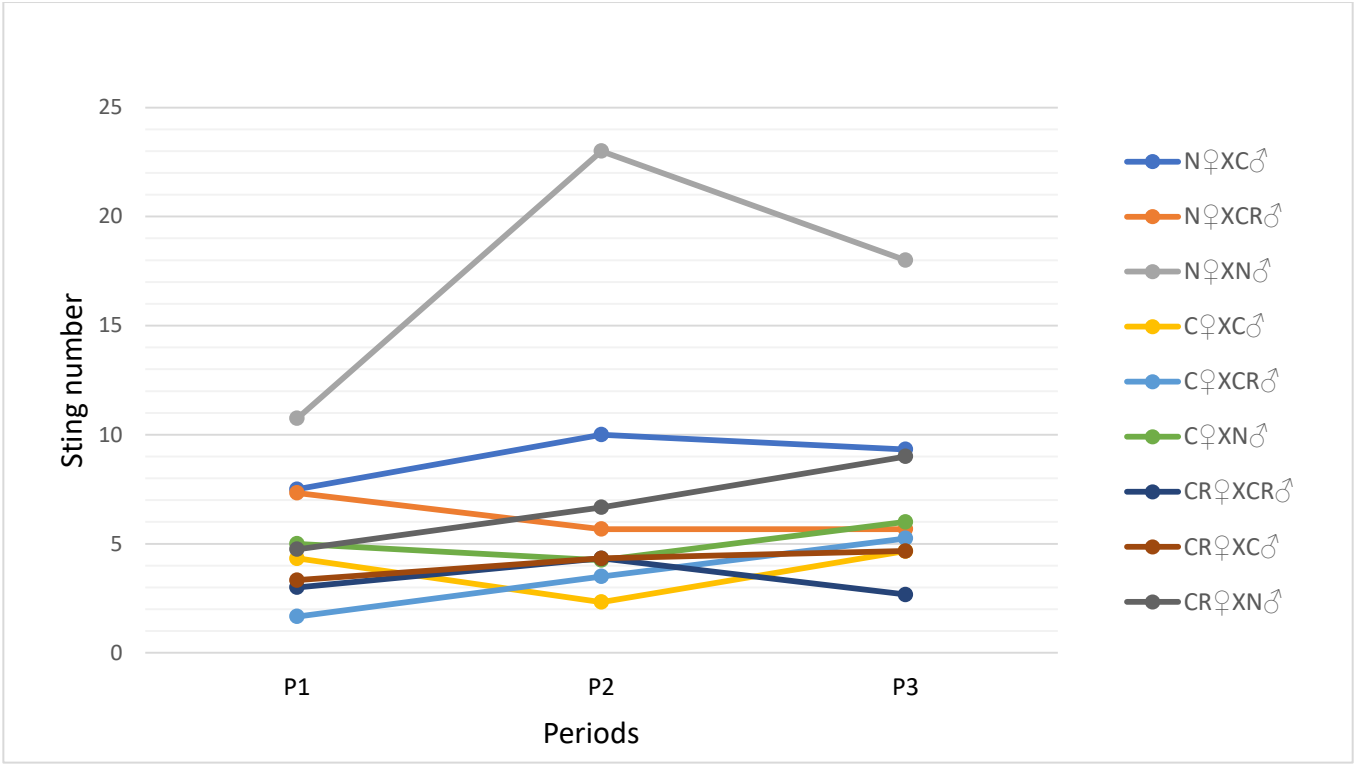

Figure 4. Number of stings (defensive behavior) according to genotype and time period.

Honey yield

Table 6 presents the honey yield results for the study groups. The highest honey yield was observed in the $CR^{♀}XN^{♂}$ group, followed, in descending order, by the $N^{♀}XN^{♂}$, $N^{♀}XC^{♂}$, $CR^{♀}XCR^{♂}$, $N^{♀}XCR^{♂}$, $CR^{♀}XC^{♂}$, $C^{♀}XC^{♂}$, $C^{♀}XN^{♂}$, and $C^{♀}XCR^{♂}$ groups. The ANOVA conducted on the honey yield data revealed that the honey yield for the study groups differed significantly ($P < 0.05$). Furthermore, the Duncan Multiple Range Test revealed that the $N^{♀}XN^{♂}$ and $C^{♀}XCR^{♂}$ groups were significantly different ($P < 0.05$).

Table 6. Honey yield (kg/colony) according to the study groups.

Genotype	n	Honey yield
$N^{♀}XC^{♂}$	2	12.00±8.00 ^{ab}
$N^{♀}XCR^{♂}$	2	7.50±0.50 ^{ab}
$N^{♀}XN^{♂}$	5	13.40±2.80 ^{ab}
$C^{♀}XC^{♂}$	3	6.00±1.16 ^{ab}
$C^{♀}XCR^{♂}$	3	4.00±1.53 ^{b}
$C^{♀}XN^{♂}$	2	5.00±2.00 ^{ab}
$CR^{♀}XCR^{♂}$	1	9.00±0.00 ^{ab}
$CR^{♀}XC^{♂}$	2	6.50±0.50 ^{ab}
$CR^{♀}XN^{♂}$	5	14.80±2.85 ^{a}

Different letters indicate significant differences ($p<0.05$).

The honey yield data for the study groups were obtained after a single harvest. The $CR^{♀}XN^{♂}$ genotype produced the highest honey yield averages (14.80±2.85 kg), followed by the $N^{♀}XN^{♂}$ (13.40±2.80 kg) and $N^{♀}XC^{♂}$ (12.00±8.00 kg) groups. On the other hand, the $C^{♀}XCR^{♂}$, $C^{♀}XN^{♂}$, and $C^{♀}XC^{♂}$ genotypes were the groups with the lowest mean values (4.00±1.53 kg, 5.00±2.00 kg, and 6.00±1.16 kg, respectively).

Figure 5 graphically presents the honey yields of the study groups. Determination of honey yield was based on the production of 2 kg of honey in each frame with bees in the colony. Accordingly, honey harvest was possible in 5 colonies in the $CR^{♀}XN^{♂}$ and $N^{♀}XN^{♂}$ groups, which finished the experiment with 7 colonies.

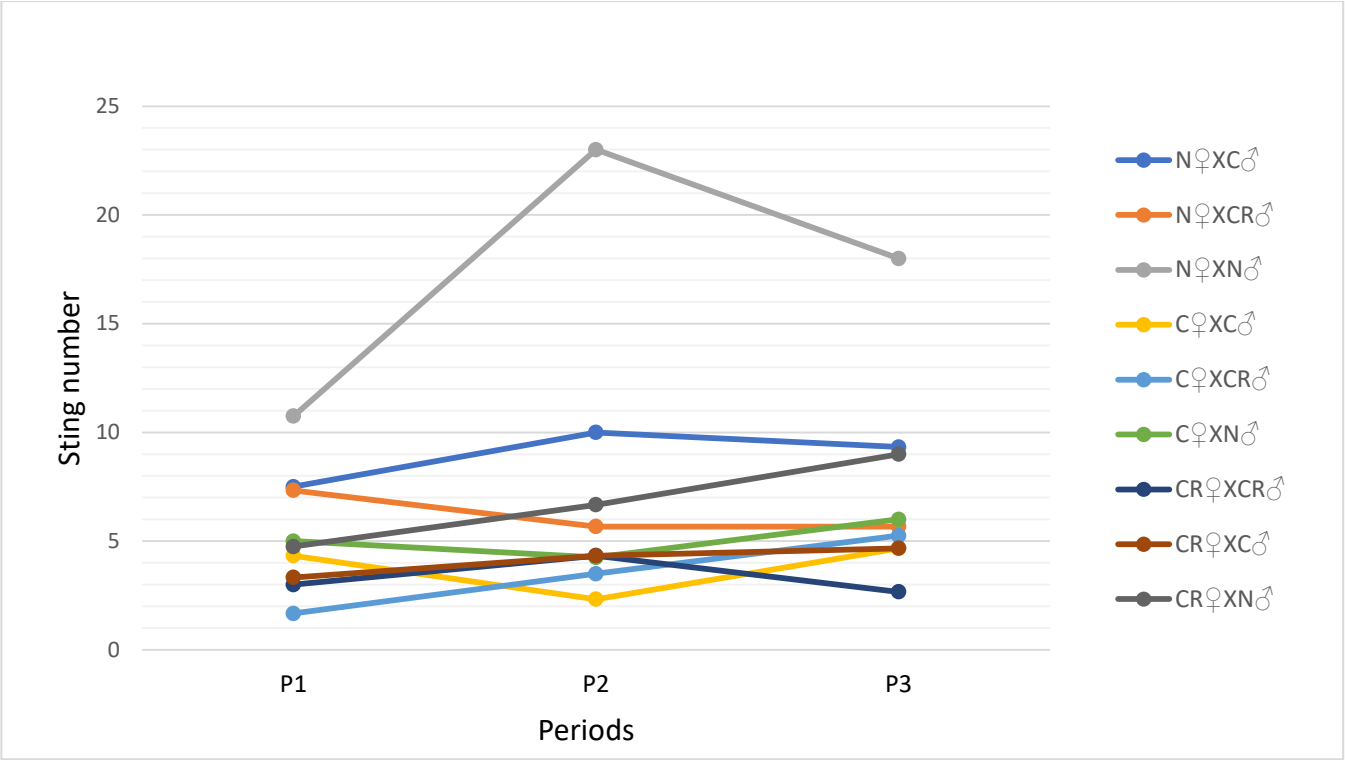

Figure 5. Honey yield (kg/colony) according to genotype

4. Discussion

In a study conducted by Gencer and Karacaoglu (2003) to compare the brooding and honey yield characteristics of the crossbreeds of Caucasian and Anatolian bees (*A. m. anatoliaca,* Aegean ecotype), it was observed that the brooding rate of the Caucasian genotype was lower than that of its hybrids. Moreover, the brooding even ceased towards the end of the production season, posing a risk to wintering. In another study by Güler and Kaftanoğlu (1999), where the performances of important honeybee breeds and ecotypes in migratory beekeeping conditions in Turkey were compared, it was reported that Caucasian genotypes had the lowest mean brooding rate.

Caucasian bees, which start winter stocking early due to their breed characteristics, tend to reduce brooding earlier compared to other breeds (Dolgiva et al. 2021). Consequently, significant colony and yield losses are observed outside their natural habitats. It was hypothesized that the rising air temperatures in the study area in mid-July could be a crucial factor contributing to the decrease in brooding. However, Carniolan and native bees, as well as cross hybrids, displayed higher averages.

Carniolian honeybees are recognized for their wintering abilities, spring development, high honey yield, and calm nature within their native ecology (Kakhramanov et al. 2021). Consequently, the demand for Carniolan bees is on the rise in various regions across Turkey. However, there is a scarcity of studies examining the performance of Carniolan bees in different geographical areas.

Although Carniolan bees are typically described as winter bees (Collison 2023), it is noteworthy that two colonies in the pure genotype group ($CR^{♀}XCR^{♂}$) were recorded as winter losses in the present study. In a study conducted by Arslan et al. (2004) in the Tokat province, the wintering capabilities of F1 hybrids formed by free breeding of Tokat, Muğla, Carniolan, Caucasian-TKV, Italian, and Caucasian-Camili genotypes were analyzed. In that study, it was determined that the group formed with Carniolan bees exhibited the highest wintering capability (Arslan et al., 2004). The discrepancy between this finding and those of the present study emphasizes the importance of conducting regional studies before drawing final conclusions.

One of the noteworthy findings of the study was that the pure native genotype ($N^{♀}XN^{♂}$) and native male hybrids ($C^{♀}XN^{♂}$ and $CR^{♀}XN^{♂}$), which have adapted to conditions where they have been bred for an extended period, ranked among the three honeybee groups with the highest brood areas. These findings

emphasize the importance of utilizing indigenous gene resources in determining suitable breeding material in various regions.

It was observed that the distribution of the groups based on the number of frames with bees, which reflects adult bee development, was similar to that associated with the production of brood area. The relatively low mean number of frames with bees, compared to that observed in previous studies (Güler and Kaftanoğlu 1999; Akyol et al. 2014) could be attributed to the overall unfavorable season for colony development and yield during which the study was conducted. However, the maximum number of adult bees observed during the nectar flow period remained consistent with the results of the studies mentioned above.

It is well-established that various factors directly or indirectly influence the defensive behavior of bees (Patrice et al. 2018; Omar, 2020). In this study, the analysis of the number of stings on the black-painted tennis balls revealed that the native bees ($N^{\varphi}XN^{\sigma}$) stood out as the most aggressive group, with a mean sting count of 16.60±2.38. Furthermore, the $CR^{\varphi}XCR^{\sigma}$ and $C^{\varphi}XC^{\sigma}$ genotypes were identified as the calmest groups, with mean sting counts of 3.33±0.47 and 3.78±0.62, respectively. These findings indicated that both the Carniolan (Cengiz and Erdoğan 2018; Abacı and Bıyık 2021) and Caucasian (Cengiz and Erdoğan 2018; Svistunov et al. 2018; Arslan 2020) genotypes, known for their calm temperament, and the native bees, known for their aggressiveness, exhibited these same traits under local conditions. However, a significant finding of the study was that the aggressive tendency, which can pose challenges in various beekeeping applications, could be improved through hybridization.

It is also well-established that numerous environmental and genetic factors can effectively influence honey yield (Guichard et al. 2023). The observation that the mean honey yield values found in the present study were lower than both the national average for Turkey (13.79 kg; Tuik 2020) and the averages reported in various studies with different genotypes (Dogaroglu et al. 1992; Gencer and Karacaoglu 2003) could be attributed to factors such as insufficient nectar resources and environmental constraints, including climatic fluctuations.

5. Conclusions

Within the limitations of the present study, which investigated the performance of pure and crossbred hybrids of three genotypes, it was observed that Caucasian and Carniolan bees, known for their superior yield in their native habitats, failed to exhibit the same level of performance under the ecological conditions of the Van region. Conversely, it was also demonstrated that the yield of native bees, which have adapted to local conditions for years, could be enhanced through selection and hybridization.

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