Bee Studies 15(1), 25-35
http://doi.org/10.51458/BSTD.2023.33

RESEARCH PAPER

Comparative Morphological Analysis of Honey Bees (*Apis mellifera* L.) from the Western and Central Black Sea Region, with Emphasis on Natural Diversity

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Article History
Received 20 April 2023
Accepted 28 July 2023
First Online 28 July 2023

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Keywords
Subspecies
Genotype
Morphology

Abstract

Due to migratory beekeeping and nationwide sales of queens and colonies, genetic mixes are a major concern. Nonetheless, providing beekeepers with quality breeding material will boost production. Two hundred colonies were chosen from the Western and Central Black Sea Region, specifically from Düzce, Kastamonu, Sinop, and Ordu. These colonies were selected from locations where migratory beekeeping is not practiced, and the beekeeping firms involved have abstained from using commercial queen bees for three generations, between 2014 and 2022. Each sample has 10 worker bees with 41 morphometric measures. The local bee genotype was compared to Caucasian, Anatolian, and Yığılca genotypes, whose comprehensive morphological traits were known. Four local bee genotypes differed significantly (P<0.05) in 39 morphological features. These four bee genotypes clustered into three function value groups. With 98.9% accuracy, the 271 worker bee samples from the four genotypes were categorized in respective fields. Anticipated were strong morphological similarities between worker bee samples from the nearby regions of Yığılca and WCBS. The Caucasian bee subspecies, along with the other three genotypes, had a significant level of morphological likeness and overlap. Selection may affect qualitative characters (color, etc.) like quantitative characters across generations. Protect, generate, and provide breeding material with better productivity, wintering capacity, and morphology.

Introduction

Honeybee (*Apis mellifera* L.) populations can be found in almost every region of the world, despite the fact that the African Continent is the species' original home. These honey bee populations have adapted to very different ecological conditions and have different structures in terms of morphological, physiological, behavioral, and molecular structure as a result of these adaptations (Alpatov, 1929; Bilash et al., 1976; Ruttner, 1988; Rinderer et al., 2010). Honey bee populations that have settled in different isolated regions have developed distinct morphological characteristics as a result of natural selection, genetic drift, and mutation (Ruttner et al., 1978; Settar, 1983; Bodur et al., 2007; Güler, 2010). These morphological differences have been attributed to natural selection, genetic drift, and mutation. Variations in populations that were established in distinct isolating zones propagated independently of one another, which resulted to the emergence of new genotypes (Alpatov, 1929). When the climate had reached a stable state, they were redistributed to their previous habitats, where they met and shared genetic material (Alpatov, 1929; Ruttner, 1988; Smith, 1991). The subspecies of the honeybee were categorized into three major lineages: A (Africa), C (Carnica), and M (Mellifera). This classification was developed by taking into consideration the physical characteristics of the honey bee (Ruttner et al., 1978; Rinderer et al., 1993; Smith et al., 1997; Palmer et al., 2000; Ruttner et al., 2000; Kandemir et al., 2000). The abbreviation of Region Honey Bees is WCBS.

Breeders don't recommend some types of bees because they don't make enough honey, swarm a lot, or are very mean. Native bee breeds were threatened with extinction as a result of the preference for alien kinds. In terms of consistency in local settings, however, European breeds fall short of breeders' goals. Since pure European-breed colonies cannot produce more honey than breeding stock under these circumstances, and because their hybrids grow more aggressive, pure European-breed colonies cannot produce more honey than breeding stock. The Western Black Sea is commonly recognized as one of the most important bee gene pools in Anatolia. Although the area lies outside of the bees' migration route, it is favorable for the preservation of genetic and breeding material. On the population's behavior, performance, and morphology, however, there is inadequate study and data. In this regard, it is unknown whether the population in the region is homogeneous, whether it is exposed to the mixture, whether it is a distinct taxonomic unit, and whether it is related to the taxonomy of races in Anatolia, such as *Apis mellifera anatoliaca, Apis mellifera caucasica, Apis mellifera carnica, Apis mellifera syriaca, Apis mellifera* (Kandemir et al., 2000; Güler, 2010)

According to Ruttner (1988), *A. m. caucasica* is found from Samsun to the north-east of Turkey, *A. m. meda* is found at Southeastern Anatolia, *A. m. syriaca* is found along the Turkish-Syrian border and in the province of Hatay *A.m. syriaca*, and *A. m. anatoliaca* Mugla honey bee genotype were found in the rest of Turkey reported in 2022.

The objective of this study is to analyze the morphological structure of the bee population in the Central and Western Black Sea Region. This population displays clear distinctions from the existing bee races in Turkey and has been the focus of a relatively little amount of research up to this point. This population is being preserved in its native territory so that it can continue to contribute to the genetic diversity of Anatolian bees and ensure the continued viability of beekeeping in the country as a whole. The research entails both the characterization of material that has been subjected to three generations of selection and the comparison of its morphological structure with bee races and genotypes that have adapted to geographical areas that are nearby.

Material and Methods

Material

The material has been utilized to produce 200 colonies, all of which were collected in 2014 from stationary apiaries located at some regions of the Western and Central Black Sea Regions. In these regions there is no practice migratory beekeeping and do not make use of commercial queens. Western and Central Black Sea Region Honey Bees is abbreviating WCBS.

Method

These colonies were chosen because the index values of the 50 colonies that formed in the 25% slice showed that they did better in terms of honey production, how well they raised their young, and how well they survived the winter. Each chosen colony was represented by four queens from the same family in each generation. In this direction, queen bees were raised every two years in May and June. Ten microliters of homogenized semen from colonies other than their own was used to artificially inseminate these queens. The colonies took the queen bees that had been fertilized, and the herd size was back up to 200. The study, which began in 2014, is now in its third generation, with a performance test every other year and a selection every other year. At the end of the third generation, worker bee samples were taken from 50 colonies during the swarming period. Morphometric measurements were taken of 10 worker bees and 41 characters in each sample (Alpatov, 1929; Ruttner et al., 1978; Güler & Kaftanoglu, 1999a; Güven, 2003).

The morphological data of 98 Anatolian and 98 Caucasian race samples, as well as 25 samples of Düzce Yığılca ecotypes, previously examined by the Faculty of Agriculture at 19 Mayıs University, were compared with 50 samples of WBCS in our study. Totally 271 samples were used. The utilization of the Anatolian race, Caucasian race, and Yığılca ecotype in the comparison is attributed to their original distribution areas of the WBCS bee.

Morphological Evaluation

The samples were collected in June, when the colonies had the young worker bees. Ten worker bees from each sample were used to collect morphometric measurements of 41 common morphological features. The morphological characters measured were the following in each worker bee: fourth tergite length of hairs (LH, mm), fourth tergite hair band width (WTa, mm), fourth tergite hair shiny surface width (WTb, mm), tomentum index (TI, ratio), length of proboscis (LPr, mm), length of femur (LF, mm), length of tibia (LT, mm), length of metatarsus (LM, mm), width of metatarsus (WM, mm), metatarsal index (MI, ratio) , hind leg length (LHL, mm), third tergite width (WT_3, mm), third sternite width (WS_3, mm), wax gland surface length (MSU, mm), wax gland surface width (WWM, mm), distance between wax surfaces (MAM, mm), sixth sternite length (LS_6, mm), sixth sternite width (WS_6, mm), sternum index (S_6I, ratio), wing length (LH, mm), wing width (KG, mm), cubital vein a length (LCa, mm), cubital vein b length (b, mm), cubital index (CI, ratio), second tergum (CT_2), third tergum (CT_3), and scutellum (CSc) colors, as well as the morphometric measurements of wing vein angles A_4, B_4, D_7, E_9, G_{18}, J_{10}, J_{16}, K_{19}, N_{23}, and O_{26} (Alpatov, 1929; Dupraw, 1965; Ruttner et al., 1978; Moritz, 1991; Kauhausenkeller ve ark., 1997; Akyol, 1998; Güler & Kaftanoğlu, 1999a; Güler, 2001; Güler & Bek, 2002; Güler et al., 2010). The measurements were measured by stereomicroscope in the morphometric measurement package.

Statistical Evaluation

In this investigation, the morphological characteristics of worker bees from four distinct genotypes were thoroughly analyzed. Single-factor analysis of variance (ANOVA) was applied to the data for 41 morphological traits to assess the variations within the categories. Subsequently, Multivariate Discriminant Analysis was utilized to ascertain the level of variation within the region. To evaluate the differences between groups, ANOVA was used to determine the mean values and standard deviations, and the DUNCAN multiple comparison test was used to compare the means. Analysing the relationships between traits, distinguishing morphological characteristics, and non-distinguishing ones was also part of the analysis. It was determined the functions representing these characteristics and their discriminate power, as well as Fisher's linear, standard, and non-standard Discriminant Function Coefficients representing the genotypes, and the Constant Discrimination Coefficients. The study also utilized Discriminant Analysis to determine if there were significant differences among the groups, analyzing variance and providing means with standard deviation. The tables displayed averages with standard deviations. Manova was also used in conjunction with Multivariate Discriminant Analysis to assess the degree of variation within characteristics.

Results

In terms of morphological measurements, comparison of the WCBS and Yığılca genotypes alone would not be adequate or correct. These two bee genotypes are thus mostly Anatolian and Caucasian based. It would be more appropriate to compare the morphological structure of the breeds. The standard morphological characteristics of 271 worker bee samples from Anatolian and Caucasian bee varieties, as well as Yığılca and WCBS bee genotypes, were investigated in this study. The data were sourced from the beekeeping unit at 19 Mayıs University Faculty of Agriculture. In Tables 1, 2, 3, and 4, the mean and

Table 1. Morphological traits (mean±sem) for honeybee genotype groups

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
LH	0.221±0.004^{c}	0.252±0.003^{b}	0.267±0.003^{a}	0.223±0.002^{c}	0.238±0.001
Wta	0.871±0.005^{b}	0.858±0.003^{b}	0.931±0.008^{a}	0.826±0.005^{c}	0.858±0.003
Wtb	0.329±0.005^{c}	0.536±0.003^{a}	0.440±0.012^{b}	0.523±0.003^{a}	0.483±0.005
LPr	6.545±0.018^{b}	6.436±0.011^{c}	6.528±0.018^{b}	6.671±0.014^{a}	6.549±0.009
LF	2.638±0.009^{b}	2.627±0.006^{b}	2.798±0.051^{a}	2,653±0.006^{b}	2.660±0.007
LT	3.273±0.010^{b}	3.098±0.006^{c}	3.429±0.013^{a}	3.299±0.008^{b}	3.241±0.008
LM	2.022±0.006^{c}	1.971±0.004^{d}	2.145±0.035^{a}	2.068±0.005^{b}	2.036±0.006
WM	1.157±0.004^{c}	1.164±0.004^{c}	1.281±0.006^{a}	1.180±0.004^{b}	1.183±0.003
W T_3	2.210±0.006^{b}	2.016±0.004^{d}	2.256±0.011^{a}	2.132±0.004^{c}	2.121±0.005
W T_4	2.154±0.007^{b}	1.982±0.004^{d}	2.199±0.014^{a}	2.086±0.005^{c}	2.076±0.005
WS_3	2.743±0.005^{c}	2.777±0.005^{b}	2.883±0.018^{a}	2.769±0.005^{b}	2.781±0 004
LWM	1.423±0.004	1.451±0.004	1.423±0.043	1.446±0.003	1.441±0.005
WWM	2.351±0.005^{b}	2.325±0.005^{b}	2.257±0.027^{c}	2.384±0.004^{a}	2.342±0.004
DWM	0.287±0.001^{c}	0.304±0.002^{a}	0.295±0.004^{b}	0.291±0.001^{bc}	0.296±0.001
LFW	9.107±0.024^{b}	8.376±0.009^{c}	9.562±0.054^{a}	9.056±0.018^{b}	8.891±0.026
WFW	3.105±0.009^{b}	2.821±0.004^{c}	3.320±0.043^{a}	3,134±0.007^{b}	3.043±0.012
LCa	0.528±0.004^{b}	0.479±0.002^{c}	0.483±0.004^{c}	0.539±0.002^{a}	0.509±0.002
LCb	0.241±0.002^{b}	0.250±0.001^{a}	0.219±0.003^{c}	0.241±0.001^{b}	0.241±0.001
LS_6	2.531±0.005^{c}	2.548±0.004^{bc}	2.671±0.012^{a}	2.557±0.005^{b}	2.564±0.004
WS_6	3.101±0.009^{b}	3.119±0.006^{b}	3.336±0.021^{a}	3.123±0.006^{b}	3.144±0.006

LH= length of hair, WTa=width tomentum a WTb= width tomentum b, LPr= Length of proboscis, LF= Length of femur, LT= Length of tibia LM = Length of metatarsus WM = Width of metatarsus, WT_3 = Width of tergite WS_3= Width of sternite LWM= Length of wax mirror, WWM= Widht of wax mirror, DWM= D between mirrörs, LFW= length of forewing, WFW=wing of forewing LCa= length of cubital a LCb= length of cubital b, LS_6= length of sternum 6 , WS_6= width of sternum 6 , WT_3+WT_4= BS body size standard error values for each group of bees studied are presented.

In a one-way variance analysis, it was determined that four regions of bees were significantly different from each other in terms of 39 morphological characters, except for the wing O_{26} vein angle and the length of the wax mirror (LWM) ($P < 0.05$). Especially when these four regional bees were characterized quantitatively or descriptively in terms of morphology, it was observed that they exhibited different structures.

In terms of WBCS, it was determined that the average largest wing B_4, E_9, and K_{19} vein angles, tomentum index (TI), cubital index (CI), sternum under index (S_6I), and second yellowest (CT_2), third (CT_3), fourth (CT_4) tergum and scutellum (CSC) color were found in WCBS genotype. On the other hand, this bee had the smallest average wing A_4, D_7, L_{13} vein angles, shortest length of hairs (LH), fourth tergum felt glossy ground width (WTb), metatarsal length (LM), metatarsal width (WM), sternum under width (WS6), wax mirror distance (DWM), sternum under length (LS_6), hind leg length (LHL) and metatarsal index (MI).

The Anatolian bee had the largest average fourth tergum felt glossy ground width (WTb), wax mirror distance (DWM), cubital b vein length (LCb), wing E_9 and L_{13} vein angles, scutellum (CSC) color and sternum under index (S_6I) value. On the other hand, it showed the smallest average length of proboscis (LPr), length of tibia (LT), metatarsal width (WM), shortest wing length

Table 2. The mean and standard error values of the vein angles A4, B4, D7, E9, J10, J16, N23, L13, K19, G12, and O26 observed in worker bee samples from four regions

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
A_4	32.087±0.177 ^{c*}	32.935± 0.134^b	34.981±0.229 ^{a}	32.529±0.104 ^{ab}	32.898±0.088
B_4	104.646±0.422 ^{a}	102.152± 0.276^b	100.528±0.566 ^{c}	102.458± 0.301^b	102.501±0.188
D_7	100.597±0.276 ^{c}	101.655± 0.216^b	102.974±0.240 ^{a}	100.880±0.175 ^{c}	101.361±0.120
E_9	20.468±0.111 ^{a}	20.416±0.111 ^{a}	20.688±0.132 ^{a}	19.341± 0.074^b	20.084±0.062
J_{10}	52.238± 0.312^b	48.499±0.205 ^{c}	56.089±0.396 ^{a}	51.927± 0.203^b	51.305±0.191
J_{16}	91.435± 0.292^b	92.128±0.222 ^{ab}	87.661±0.348 ^{c}	92.297±0.229 ^{a}	91.507±0.157
L_{13}	14.430±0.095 ^{c}	17.774±0.097 ^{a}	14.344±0.204 ^{c}	15.304± 0.099^b	15.890±0.102
N_{23}	89.415± 0.289^b	88.520± 0.281^b	84.243±0.634 ^{c}	93.581±0.228 ^{a}	89.911±0.241
K_{19}	76.965±0.224 ^{a}	75.680± 0.195^b	76.288±0.307 ^{ab}	76.351±0.177 ^{ab}	76.218±0.110
G_{12}	92.010± 0.277^b	88.477±0.223 ^{c}	95.921± 0.394^a	91.968± 0.173^b	91.250±0.186
O_{26}	34.952±0.302	36.052±0.191	37.497±0.334	35.871±0.234	35.973±0.131

*=different letters indicate different averages

(LFW), cubital a vein length (LCa), wing J_{10} vein angle, and third tergite color (CT_3).

Among the genotypes, the Caucasian bees displayed the greatest diversity in measured morphological characteristics compared to other bee breeds, with the highest average values. This bee showed the longest hair (LH), the largest fourth tergum velvet ground width (WTb), length of femur (LF), length of tibia (LT), metatarsal length (LM), metatarsal width (WM), the widest width of tergite fourth tergum width (WT_3 and WT_4), third sternum width (WS_3), wing length (LFW), sternum lower length (LS_6), sternum lower width (WS6), the largest wing vein angles A_4, D_7, E_9, J_{10}, G_{12}, O_{26} , the largest hind leg length (ABU), the largest body size (WT_3+WT_4=BS), cubital index (CI) and metatarsal index (MI) (Tables 1, 2, 3, and 4). Additionally, it exhibited the lowest average values for wax gland surface width (WWM), cubital vein lengths a and b, the smallest wing vein angles B_4, J_{16}, L_{13}, N_{23}, fourth tergum color (CT_4), and the most yellow scutellum color (CSC). As seen, the Caucasian bee breed exhibited the longest hair (LH), the largest wing vein angles, the darkest color, the largest body, the longest hind leg, and the largest cubital (CI)

Table 3. Mean and standard error values of the second, third tergite and scutellum colors (scale) of worker bee samples from four regions

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
CT_2	7.330±0.125 [a]	6.539± 0.104[b]	5.380±0.177 [d]	5.793±0.085 [c]	6.275±0.067
CT_3	7.125±0.066 [a]	5.765±0.065 [c]	5.165± 0.199[d]	6.430± 0.074[b]	6.164±0.056
CT_4	4.438±0.059 [a]	2.859± 0.050[b]	2.609± 0.196[c]	4.257±0.069 [a]	3.596±0.060
CSc	2.337±0.096 [a]	2.207±0.094 [a]	0.376±0.066 [c]	1.226± 0.066[b]	1.660±0.060

CT_2 = color of second tergite, CT_3= color of third tergite, CT_4 = color of fourth tergite, CSc = color of scutellum, [*]= different letters indicate different averages

and metatarsal indices among the morphological structures.

The Yığılca bee genotype exhibits the highest values for average length of proboscis (LPr), fourth tergite wax patch width (WTb), wax gland surface area (WWM), cubital vein A1 length, wing vein angles J_{16} and N_{23}, fourth tergite color (CT_4), cubital index (CI), and sixth sternum index (S_6I). It has the shortest length of hairs on average (LH), the smallest fourth tergite wax band width (WTa), third (WT_3) and fourth (WT_4) tergite widths, the yellow second tergite color (CT_2), tomentum index (TI), the small body size (BS), and the small metatarsal index (MI).

Table 4. Mean and standard error values of tomentum, cubital and metatarsal index (ratio) and hind leg length (mm) determined by calculating worker bee samples from four regions

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
TI	2.819±0.068 [a]	1.654±0.018 [c]	2.253± 0.048[b]	1.612±0.015 [c]	1.922±0.031
LHL	7.934± 0.024[c]	7.698±0.015 [d]	8.373±0.077 [a]	8.022± 0.019[b]	7.937±0.018
BS	4.365± 0.012[b]	3.999±0.007 [d]	4.456±0.024 [a]	4.219±0.009 [c]	4.198±0.011
CI	2.227±0.033 [a]	1.958± 0.016[b]	2.214±0.028 [a]	2.266± 0.021[a]	2.145±0.014
MI	57.251± 0.177[c]	59.155± 0.220[b]	60.740±1,815 [a]	57.087± 0.144[c]	58.293±0.255
S_6 I	81.709±0.232 [a]	81.773±0.132 [a]	80.126± 0.243[b]	81.929±0.136 [a]	81.611±0.090

TI = tomentum index, LHL = hind leg length, BS($T_3 + T_4$)= body size, CI = cubital index, MI = metatarsal index, S_6 I = sixth sternum index, [*]= different letters indicate different averages.

In general, the WCBS breeding material showed similarity with the Anatolian bee race in 10 (WTa, LT, WM, WWM, WS_6, E_9, J_{16}, N_{23}, CSC, and S_6I) morphological characters, with the Yığılca genotype in 12 (LH, LT, LFW, a, D_7, J_{10}, G_{12}, CT_4, CI, MI, and S_6I) characters, and with the Caucasian bee race in 4 (LPr, E_9, L_{13}, and CI) characters.

In addition, multivariate discriminant analysis was carried out on 41 morphological characteristics of 271 worker bee samples that were representative of the four different bee genotypes. In the process of grouping, all of the other 40 characters, with the exception of the O26 character, were determined to be significant ($P<0.001$). In addition, the body size measure, also known as the BS character, was not taken into account in the discriminant analysis that was performed on the sample grouping, which was dependent on the tolerance test.

The application of Linear Discriminant Analysis to morphological characteristics led to the determination of the number of functions responsible for the grouping, as well as their appropriate values, variance levels, Wilks' lambda, and significance levels. Table 5 contains the values related to these factors, and the table itself can be found here.

In addition, the discriminant function represented by each morphological character or the characters that

Table 5. The values for the fitness values, variance, total variance levels, WilksLambda values and significance levels of the grouping functions

Function	Fitness Values	Variance (%)	Total Variance (%)	Wilks lambda	*P*
1	117,637 [a]	79.00	79.00	0.000	0.000
2	24,120 [a]	16.20	95.30	0.005	0.000
3	7.064 [a]	4.70	100.00	0.124	0.000

a : The first three discriminant functions were found to be successful in grouping.

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Table 6. Morphological characters describing grouping functions and their related structure matrix

Morphological character	Function		
	1	2	3
LT	.656 *	.264	.188
LPr	-.066 *	.058	-.007
E_9	.056 *	.019	.028
LH	-.073	.403 *	.303
WT3	-.026	.363 *	.139
WT3+ WT4(BS)	-.024	.337 *	.133
WTb	-.055	-.321 *	.226
L_{13}	.049	-.282 *	-.040
TI	-.218	.281 *	-.199
WT_4	-.019	.270 *	.111
WFW	-.064	.268 *	.231
CI	-.044	.104 *	.004
DWM	.017	-.063 *	.034
K_{16}	-.006	.047 *	-.033
MSU	-.003	-.024 *	-.003
O_{26}	.004	-.014 *	.000
WS6	.016	.076	.324 *
WM	.002	.078	.316 *
CSC	.041	-.061	-.275 *
LS_6	.006	.055	.275 *
CT_4	-.069	.102	-.269 *
CT_3	-.026	.067	-.265 *
A_4	.020	.018	.245 *
WS3	.013	.027	.242 *
G_{12}	-.030	.199	.220 *
ABU	-.029	.140	.212 *
J_{10}	-.026	.200	.210 *
CT_2	.035	.010	-.210 *
N_{23}	-.093	-.044	-.207 *
LFW	.043	-.041	.193 *
J_{16}	-.022	-.082	-.187 *
LCa	-.080	.092	-.169 *
LM	-.028	.088	.154 *
LCb	.003	-.086	-.146 *
B_4	.000	.027	-.140 *
LT	.002	.047	.139 *
WTa	.043	.064	.137 *
WWM	-.038	-.015	-.134 *
D_7	.017	-.005	.129 *
S_6I	-.014	-.040	-.120 *
MI	.020	-.010	.087 *

are effective in determining the function were determined and presented in Table 6.

Length of tibia (LT), length of proboscis (LPr), and wing E_9 vein angle characters were represented by the first function (F1), while length of hairs (LH), third tergite width (WT_3), body size (BS), fourth tergite shiny ground width (WTb), wing L_{13}, K_{16} vein angles, tomentum index (TI), fourth tergite width (WT4), wing width (WFW), cubital index (CI), and distance between wax glands (DWM) were represented by the second function (F2), and the other 25 characters were represented by the third function (F3). These three functions fully grouped the 271 worker bee samples representing the four regions. However, the first discriminant function alone could explain 79.00% of the total variance. The discrimination power of the first function can also be seen in the Wilks Lambda (0.000) value it received. As the WilksLambda value, which describes the power of a function in grouping, approaches 0, the discrimination power increases, which is easily seen in this study (Coley & Lohnes, 1971; Le, 2001). Moreover, this function achieved this discrimination power with only three morphological characters (LT, LPr, and E9) (Table 6). On the other hand, the WilksLambda value of the third function was low (0.124) and showed a grouping power of only 4.7% of the total variance. Therefore, it can be said that three functions are sufficient to group the samples. Indeed, the first and second functions explained 95.3% of the total variance. This result also shows that there is significant variation among genotypes in terms of their morphological structure (Güler & Kaftanoğlu, 1999b; Güler, 2010).

When the one-way analysis of variance (ANOVA) results from these three characters representing the discriminant function are examined, it is seen that the Caucasian bee has the longest length of tibia (LT) and the largest wing E_9 vein angle, while the Anatolian bee has the shortest length of proboscis and smallest tibia length.

The Anatolian, Caucasian bee races, WCBS and Yığılca genotypes were illustrated with 98, 35, 50 and 98 samples, respectively, in the group of 271 worker bee samples belong to four genotypes and evaluated using the Discriminant analysis method. The results of demonstrating the real or closest related groups and overlapping levels of the original samples are given in Table 7.

Table 7. The original and second most reliable predicted grouping numbers and ratios of 271 worker bee samples representing Anatolian, Caucasian bee breeds and WCBS and Yığılca genotypes

Original Regions	Estimated group membership			
	WCBS	**Anatolia**	**Yığılca**	**Caucasian**
WCBS	50 (100%)	0	0	0
Anatolia	0	98 (100%)	0	0
Yığılca	0	0	98 (100%)	0
Caucasian	2 (5.7 %)	1 (2.9 %)	1 (2.9 %)	31 (88.6 %)

The correct grouping levels of the original samples are 98.9%.

According to the discriminant analysis results of the samples representing the groups and the evaluation of their representation, discrimination, and similarities to their original groups, all 50 samples representing the WCBS genotype, 98 samples representing the Anatolian bee race, and 98 samples representing the Yığılca bee genotype have been grouped with 100% accuracy in their respective original groups. On the other hand, out of the total 35 samples representing the Caucasian bee race, two overlapped with the WCBS genotype, one with the Anatolian genotype, and one with the Yığılca genotype. Thirty-one samples represented the original Caucasian population. In this study, the Caucasian bee race showed morphological similarities of 5.7%, 2.9%, and 2.9% with the WCBS, Anatolian, and Yığılca bee genotypes, respectively.

The relationships, kinships, sharing areas, and which genotype formed the center group of the 271 worker bee samples representing these genotypes were determined in terms of their morphological structures, and the findings are presented in Figure 1.

Morphological difference according to discriminant functions (F1 and F2) is significant between

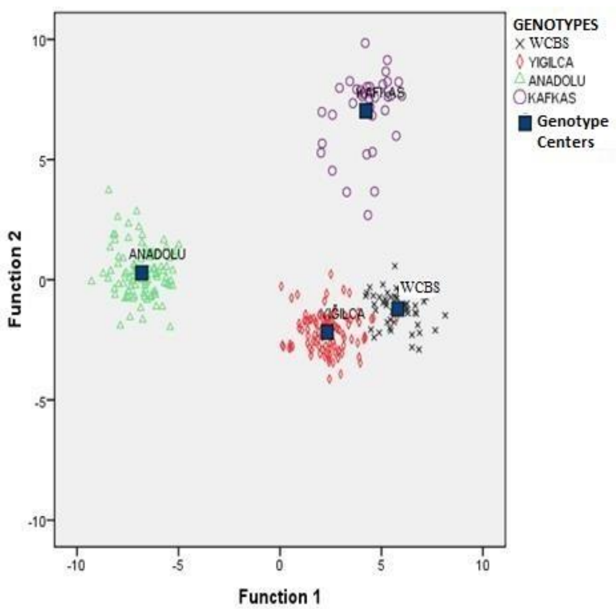

Figure 1. Distribution areas of 271 worker bee samples representing Anatolian, Caucasian bee breeds and WCBS and Yığılca genotypes in dimensionless measurement environment

all four bee genotypes (Table 7). In general, three different clustering areas depending on the size were formed in the coordinate system according to the function values. The first area of sharing was the Anatolian race, the second area was Yığılca and WCBS, and the third area was the Caucasian bee race. As can be seen in the dimensionless measurement ratio, Anatolian bee breed samples shared almost the same and narrow area in the coordinate system.

Discussion and Conclusion

Material obtained from different areas of the Central and Western Black Sea Region and subjected to three generations of selection was compared with Caucasian, Anatolian, and Yığılca bee races and genotypes in terms of 41 morphological characters. Statistical evaluation was performed using both one-way (randomized complete block design) and multivariate Discriminant Analysis, and it was observed that races and genotypes exhibited significantly different morphological structures in terms of all 39 characters except for LWM and wing O26 vein angle. On the other hand, three canonical discriminant functions correctly classified 271 samples belonging to 4 bee genotypes with a 98.9% accuracy rate. This level of discrimination power indicates that there is a significant genetic variation in terms of morphology. Indeed, it is seen that almost all of these races and genotypes are clearly separated from each other, and very low levels of overlapping sample matches occur in their distribution areas (Table 7).

The WCBS genotype is featured as the most yellow bee in terms of all color values (CT_2, CT_3, CT_4, and CSc) among the genotypes. The most yellow-colored bees distributed in Anatolia are the Syrian bee *(A. m. syriaca)* distributed in Southeast Anatolia and Anatolian bee races distributed in Central Anatolia (Ruttner et al., 1978; Akyol, 1998; Güler & Kaftanoğlu, 1999a; Gencer & Fıratli, 1999; Güler et al., 2012). Here, the WCBS exhibited a more yellow color than the Anatolian bee race except for Scutellum color. There is only a Scutellum color similarity between the Caucasian bee and the WCBS. However, in previous studies (Güler & Kaftanoğlu, 1999b; Güler & Toy, 2008; Güler et al., 2012), it was reported that Caucasian bees and Black Sea Region bees generally showed a dark color. In honey bees, the normal color of chitin is black, and this color is dominant over the yellow chitin color, which is recessive in effect. This dominant gene suppresses the effect of the recessive gene (Collins, 1986; Rinderer, 1986; Güler, 2017). Therefore, we estimate that the reason for the yellow color of the WCBS breeding material is due to the application of 3 generations of selection. It is known that the selection of behavior and performance traits of the breeding material was carried out using the index method. Therefore, the presence of yellow-colored colonies in the population and their selection as parents due to their high average index values increased the frequency of the recessive yellow color in the population over generations. It is thought that the color will become stabilized with one or two more generations of selection.

The WCBS genotype, after three generations of selection found to be similar to the Caucasian race in terms of wing vein angles (LPr), E_9 and L_{13}, and cubital index (CI), to the Anatolian race in terms of fourth tergum felt surface width (WTa), length of femur (LF), metatarsal width (WM), wax gland surface width (WWM), sixth sternum width (WS6), E_9 and N_{23} wing vein angles, scutellum color (CSc), and sternum bottom index (S_6I), and to the Yığılca genotype in terms of length of hairs (LH), length of femur (LF), length of tibia (LT), wing length (LFW), wing width (WFW), cubital B vein length, sternum bottom width (WS_6), D_7, J_{10}, G_{12} wing vein angles, fourth tergum color (CT4), cubital index (CI), metatarsal index (MI), and sixth sternum index (S_6I) characters. It can be seen, the highest morphological similarity was found with the Yığılca genotype. Although there is no overlap between the samples of these two bee genotypes, it can be said that they share the same area. The WCBS genotype was found to be similar to the Caucasian race in 4 characters, to the Anatolian race in 9 characters, and to the Yığılca genotype in 14 characters. Thus, the highest morphological similarity was shown with the Yığılca genotype (Table 1, 2, 4). However, as explained above, there is no similarity in terms of color. The question of why there are no color similarities if some of the colonies, which are breeding materials, was initially taken from Yığılca. As explained above, and the most important finding of this study. It is estimated that selection had caused significant changes in the population in terms of qualitative and quantitative characters throughout the generations.

Virtually all (98%) of the 27 bee breeds historically characterized morphologically in the globe have been adapted to a geographical region and named after that region (Ruttner et al., 1978; Ruttner, 1988). According to several scholars, the location where this study material is disseminated is the distribution area of the Caucasian bee race (Bodenheimer, 1942; Ruttner et al., 1978; Adam, 1983). According to Ruttner (1988), the Caucasian bee breed is found across the Northeast Anatolian Area, including Samsun. Yet, it is evident that the geographical structural difference within the area produces the ecological difference, i.e., the morphological differences in the bee populations. As shown in Table 7, it is estimated that the discriminant analysis approach contributes to this degree of discrimination. As in this research, when the 4 bee genotypes are examined at this level in terms of several characteristics, such as 35 to 98 colony repetitions, 10 worker bee replications in each sample (colony), and 41 characters, the approach demonstrates the ability to categorize and detect distinctions. In fact, significant morphological similarities were anticipated between neighboring worker bee samples from Yığılca and WCBS. The Caucasian bee race and the other three genotypes had the greatest degree of physical resemblance and overlap. In other words, only Caucasian samples exhibited overlap. It has been estimated that this likeness or overlap is the result of unregulated sales of this bee to almost every region of the nation over the last 35 to 40 years, as opposed to a physical similarity resulting from the original genetic structure. In reality, as stated by Güler (2010), the morphological structure of a native (Sinop-Türkeli) bee is susceptible to modification as a result of the usage of queen bees, particularly of Caucasian origin.

When certain traits of four genotypes were compared, the length of length of proboscis was found to be the biggest difference. Even though Yığılca has the longest length of proboscis structure (6.671±0.014a) and Anatolian bee has the shortest (6.436±0.011c). All of the samples from the four genotypes have a long length of proboscis structure at general. In many past studies (Alpatov, 1929; Ruttner, 1988; Oztürk, 1990; Güler &Kaftanoglu, 1999a), Caucasian bees was said to have the longest length of proboscis of the bees at that area. However, the results of this study showed that the Yığılca bee has a length of proboscis structure that is longer than that of the Caucasian bee. This study found that the average length of a Caucasian bee's length of proboscis is 6.528±0.018b mm, which is shorter than the lengths reported by Alpatov (1929) and Bodenheimer (1942) for Kars bee (6.642 and 6.645 mm, respectively) and by Güler and Kaftanoglu (1999b) for Ardahan Posof bee (6.657±0.015 mm). On the other hand, it is smaller than what Alpatov (1929) and Bilash et al. (1976) reported for the Grey Caucasian bee (6.5-6.8 and 6.7-7.20 mm, respectively). These finding fits with the Rench rule, which says that leg length, wing length, and length of proboscis are all shorter in populations that live at high altitudes. More importantly, Güler and Kaftanoglu (1999a, 1999b), and Güler and Bek (2002) said that the wing A_4 vein angle is the most important morphological characteristic of the Caucasian bee, and that the average is 34 degrees or higher. Ruttner (1988) said that the metatarsal index (MI) values of populations (Trans-Caucasian) distributed in Caucasus are higher than the average of 57.00, and this is In this study, the MI values of all four genotypes tested, including the Anatolian bee (59.155±0.220b) and the WCBS genotype (57.251±0.177c), are greater than 57.00% and have an average of 58.293±0.255. On the other hand, the A4 wing vein angle character did not have the same structure as the metatarsal index character, and the average structure of the Caucasian bee was 34.981±0.229a, which was different from the structure of the metatarsal index character. So, the A4 wing vein angle is the most important way to tell a Caucasian bee apart based on its shape.

Ruttner (1988) and Adam (1983) say that the A. m. caucasica has the largest body size of all the bee races and ecotypes that live in different parts of the Middle East. In Turkey, morphological studies (Akyol, 1998; Güler & Kaftanoglu, 1999a; Güler, 2001) found that these local bees had the biggest bodies (4.499, 4.529±0.045, and 4.53±0.015 mm) as a genotype. Alpatov (1929) and Ruttner (1988) said that Grey Caucasians had bodies that were 4.485±0.005 and 4.547 mm long, respectively. In this study, the Anatolian race had the smallest body size (3.999±0.007d), and the Caucasian race had the largest body size (4.456±0.024a).

The differences in a lot of traits are thought to be caused by important things like the altitude, ambient temperature, flora resources and diversity, and natural harmful populations of this region, where these four bee genotypes have been adapting for thousands of years, as well as the breeding period, ambient temperature during the pupa period, the age of the comb used, which affects the size of the brood cells, and the nutrition of the brood. Because of this, it is likely that there will be a difference between a bee population that has adapted to sea level and one that has adapted to an altitude of 2000–2500 m. In fact, Alpatov (1929) said that he found five different ecotypes of the Caucasian bee race in Skorikove at different altitudes ranging from 100 to 1800 m based on body size, length of proboscis length, length of hairs, tomentum index, and color. In the same way, Smith found three bee races (*A. m. litorea, A. m. scutella, and A. m. monticola)* in East Africa (Tanzania) up to an altitude of 3000 m and over a distance of 300 km (Ruttner et al., 1978). Because of this, the differences between these four genotypes, especially between the Caucasian and Anatolian bee races, should be seen as normal in this study. The most important thing we learned from this study, in our opinion, is that traits related to behavior and productivity can also cause significant changes in morphology (Rinderer, 1986; Bienefeld et al., 2007; Güler et al., 2022).

Ethical Statement

Not applicable.

Funding Information

This study is a part of the “Batı ve Orta Karadeniz Bal arılarının Islahı” (Breeding of Western and Central Black Sea Honeybees) project and supported by General Directorate of Agricultural Research and Policies, with the TAGEM/HAYSÜD/Ü/19/A4/P4/2282 project number.

Conflict of Interest

The authors declare there are no conflict of interest.

Author Contributions

Author 1: Supervision, Project Administration, Writing – Original Draft
Author 2: Project Administration
Author 3: Project Administration, Data Curation,
Author 4: Writing – Review & Editing, Formal Analysis
Author 5: Investigation
Author 6: Formal Analysis
Author 7: Writing – Review & Editing
Author 8: Investigation
Author 9: Investigation

Author 10: Investigation
Author 11: Investigation
Author 12: Methodology, Formal Analysis the final version of this manuscript. All authors approved the final manuscript.

Acknowledgements

This study is a part of the "Batı ve Orta Karadeniz Bal arılarının Islahı" (Breeding of Western and Central Black Sea Honeybees) project and supported by General Directorate of Agricultural Research and Policies, with the TAGEM/HAYSÜD/Ü/19/A4/P4/2282 project number.

Part of this study was presented as a poster in 2022 Apimondia International Congress, İstanbul, Türkiye.

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