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Microsatellite-Based Genetic Characterization of Honeybees from the Ordu Region, Türkiye

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Abstract: This study aimed to characterize the genetic structure of the honey bee (*Apis mellifera* L.) population in Ordu Province, Türkiye, using microsatellite markers and to compare it with five regional reference populations. Worker bees were sampled from 50 colonies in 2023 and genotyped at fifteen polymorphic microsatellite loci. Genetic diversity parameters, including allele numbers, heterozygosity levels, polymorphic information content, genetic distances, and molecular variance, were calculated. The Ordu population exhibited high allelic diversity (mean observed allele number= 14.0; mean effective allele number= 9.11) and high marker informativeness (mean polymorphic information content= 0.8276). The mean expected heterozygosity (0.8445) exceeded the observed heterozygosity (0.5920). Pairwise genetic distances between Ordu and other populations ranged from 1.96 to 2.62, indicating marked genetic differentiation. Molecular variance analysis showed that 19.09% of total genetic variation was attributed to differences among populations. Multivariate and clustering analyses consistently demonstrated that the Ordu population formed a distinct genetic cluster. These findings suggest that the Ordu honey bee population represents a regionally differentiated genetic unit within the Anatolian honey bee lineage, emphasizing the importance of regulated queen exchange and region-based conservation strategies.

Keywords: Allelic diversity, heterozygosity, AMOVA, Nei's genetic distance, regional gene

1. Introduction

The honey bee (*Apis mellifera* L.) occupies a central role in both the maintenance of natural ecosystems and the continuity of agricultural production due to its indispensable function in the pollination of flowering plants (Gallai et al., 2009). In addition to the production of valuable hive products such as honey, wax, pollen, royal jelly, and propolis, honey bees play a dominant role among managed pollinators in agricultural systems. Recent global assessments estimate that insect-mediated pollination services contribute over USD 800 billion annually to the world economy, underscoring the critical economic importance of pollinators in supporting global food security (Gebremedhn et al., 2025). However, over the past decades, multiple stressors-including habitat loss, pesticide exposure, pathogens, and climate change-have led to genetic erosion and the decline of local variants in honey bee populations (Goulson et al., 2015).

Although various taxonomic revisions have been proposed for honey bees, genetic, morphometric, and ethological evidence consistently supports the existence of four main evolutionary lineages within *A. mellifera*: the African, Western and Northern European, Southeastern European and Middle Eastern lineages (Ruttner, 1988; Arias et al., 2006; Bouga et al., 2011; Nawrocka et al., 2018). The phylogenetic divergence of these lineages has been shaped by a combination of geographical isolation and

environmental adaptation, resulting in distinct patterns of ecological tolerance, behavior, and morphology. *A. mellifera* naturally occurs across a vast area encompassing Europe, Africa, and Western-Central Asia. The species' limited flight range and the presence of small, isolated populations have facilitated the evolution of approximately 30 subspecies, each exhibiting distinct genetic differentiation (Ruttner, 1988), primarily driven by ecological selection pressures and biogeographical isolation (Oleksa and Tofilski, 2015).

Today, however, extensive queen trade, migratory beekeeping, and the widespread use of non-local genotypes are reversing these natural evolutionary processes, leading to increased gene flow and the homogenization of genetic structures among populations (Bouga et al., 2011). Despite this global trend, the Anatolian Peninsula remains one of the few regions where natural variation and local lineages of *A. mellifera* have been largely preserved. Situated at the intersection of four evolutionary lineages, Türkiye serves as a biogeographical transition zone and ranks among the countries with the highest levels of genetic diversity in honey bees. Five subspecies-*A. m. anatoliaca*, *A. m. caucasica*, *A. m. meda A. m. syriaca*, and *A. m. carnica*-occur naturally across the country, collectively representing nearly 20% of all known *A. mellifera* subspecies worldwide (Kandemir et al., 2000; Bodur et al., 2007; Özdil et al., 2009). The complex topography of Anatolia, its climatic transition zones, and its long geological isolation have all contributed to this remarkable genetic diversity (Ruttner, 1988; Kandemir et al., 2006).

This diversity extends beyond the subspecies level; within *A. m. anatoliaca*, numerous locally adapted variants (ecotypes) have evolved in response to environmental heterogeneity. For example, populations adapted to the hot–arid conditions of the Aegean region or the humid microclimate of the Eastern Black Sea exhibit a broad spectrum of genetic variation within the *A. m. anatoliaca* lineage (Kandemir and Kence, 1995; İvgin Tunca, 2009; Özdil et al., 2009). This genetic mosaic positions Türkiye not only as a beekeeping nation but also as a global hotspot for honey bee evolution and adaptation. Yet, this genetic wealth is increasingly threatened by anthropogenic pressures. Unregulated queen trade, migratory beekeeping, and extensive hybridization undermine the genetic integrity of local populations, making the preservation of pure genetic pools increasingly challenging (Bouga et al., 2011; Yıldız et al., 2023). The resulting rise in regional gene flow erodes the uniqueness of local ecotypes and disrupts genetic structures shaped by historical selection. For instance, Kırpık et al. (2010) reported that only 26.5% of colonies in the Kars Plateau represented pure *A. m. caucasica* ancestry, with the remainder showing hybrid composition. Similarly, Kuvancı et al. (2023) found that morphological traits in Western and Central Black Sea populations have converged toward the Caucasian bee, largely due to increased queen exchange. These findings underscore that although Türkiye retains high genetic potential, this diversity is rapidly eroding, making the implementation of strategic conservation measures urgent and indispensable.

In this context, the present study investigates the genetic structure of the Ordu honey bee (*A. mellifera*) population using fifteen polymorphic microsatellite loci and compares it with five regional reference populations representing different areas of Türkiye. By assessing levels of genetic diversity, population differentiation, and phylogenetic relationships, this study aims to clarify the genetic distinctiveness and regional positioning of the Ordu population within the broader evolutionary landscape of *A. mellifera* in Türkiye. The findings are expected to provide an empirical basis for the conservation of local genetic resources, the development of regionally informed breeding strategies, and the advancement of sustainable beekeeping practices.

2. Materials and Methods

2.1. Sample collection and comparative material

A total of 50 worker bees of *Apis mellifera* were sampled, with one worker collected from each of 50 different colonies maintained by the Ordu Beekeepers' Association Breeding Unit. Each colony was represented by a single individual to avoid pseudoreplication, and all individuals were treated as independent samples reflecting the genetic structure of the local population. To assess inter-population differences on a national scale, genotypes obtained from the Ordu population were compared with previously published data (Karabağ et al., 2020) from five local honey bee populations genotyped using the same 15 microsatellite loci: Hatay (*A.m. syriaca*), Muğla (*A. m. anatoliaca*, Muğla ecotype), Düzce (*A. m. anatoliaca*, Yığılca ecotype), Kırklareli (*A. m. carnica*), and Artvin (*A. m. caucasica*). This comparative framework enabled a standardized and comprehensive evaluation of the Ordu population's genetic profile relative to regional reference populations.

2.2. Total DNA isolation

Total genomic DNA was extracted using the High Pure Polymerase Chain Reaction (PCR)

Template Preparation Kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol. Two worker bees per colony were homogenized in 1.5 mL sterile microcentrifuge tubes to obtain 25-50 mg of tissue. Samples were mixed with 200 µL of lysis buffer and 40 µL of diluted Proteinase K, then incubated at 55 °C for 1 h. Binding buffer and isopropanol were added, and DNA purification was performed using High Pure filter columns following the manufacturer's instructions. Final elution was carried out with 200 µL of pre-heated (70 °C) elution buffer, and DNA samples were stored at -20 °C. DNA purity and concentration were determined using a BioDrop Duo spectrophotometer, and integrity was verified by agarose gel electrophoresis (BioDrop Inc., Cambridge, UK).

2.3. Microsatellite amplification

Fifteen microsatellite loci previously reported to exhibit high polymorphism among honey bee genotypes and to reliably assess population-level genetic diversity were used (Solignac et al., 2003). Loci were selected based on their polymorphic nature, multiplex compatibility, and chromosomal representation, providing a robust assessment of genetic structure. The same primer set had been successfully used by Yıldız et al. (2025) to integrate morphometric and molecular data and was shown to have high discriminatory power among *A. mellifera* subspecies. Primers were grouped into five multiplex sets, as detailed in Table 1.

Polymerase Chain Reaction were performed in 25 µL total volume using Xpert Fast Hotstart Mastermix (GRiSP, Porto, Portugal) containing 12 µL Mastermix, 2 µL DNA (50 ng μL^{-1}), 3 µL primer mix, and 8 µL ultrapure water. Amplification was carried out in a Thermo Scientific Arktik Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) under the following conditions: Initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 20 s, 54-58 °C for 25 s, 72 °C for 60 s; final extension at 72 °C for 5 min; and hold at 4 °C. Polymerase Chain Reaction products were verified by electrophoresis on 2% agarose gels in 1× TAE buffer stained with ethidium bromide at 80 V for 1 h.

2.4. Fragment size analysis

Fragment length determination was performed using a high-resolution capillary electrophoresis system (Fragment Analyzer™, Agilent Technologies, Santa Clara, CA, USA) with the dsDNA 905 Reagent Kit according to the manufacturer's instructions. Each sample (3 µL PCR product) was mixed 1:1 with denaturation buffer, heated at 95 °C for 5 min, and cooled for 3 min at room temperature. Samples were loaded into 96-well microplates with a DNA size standard (1-500 bp) for calibration. Electrophoresis parameters were optimized using the ProSize® 2.0 software. Fragments were automatically detected and sized based on peak profiles. Fragment lengths (bp) were exported, low-quality signals and ambiguous calls filtered, and validated genotypes used in subsequent population genetic analyses.

Table 1. Multiplex primer groups and descriptive information used in the PCR analyses (Solignac et al., 2003)

Multiplex groups	Primer	Annealing temperature (°C)	Product size (Bp)	Forward primer (5'-3')	Reverse primer (5'-3')
Group 1	Ap223	58	169	TCGTACAACGTCGCGCAA	GCCGCTCGCCTGTATCTG
	Ap238		251	GTCTCGTGCGTGCGAATG	TTCATCATGTTCTCAAATTTCTTTGT
	Ap273		108	GATCTTGTGTTAAACAGCCG	GATCTCTGGCAGACGAAGAG
Group 2	Ap243	58	260	AATGTCCGCGAGCATCTG	TGTTTACGAGAATTCGACGGG
	Ap085		196	GATCAAACACACAAACGAAAGC	ACCGGAAGCCTAATCAAGG
	Ac011		127	CTTACGCCAATCTCTCCACG	CGGTTAATTTCGTTTCTCGC
Group 3	A113	58	220	CTCGAATCGTGGCGTCC	CCTGTATTTTGCAACCTCGC
	At003		199	GATCATTTCTTTCATTCTTCTCTCTC	ATGCTCGACTATTCCGCG
	A028		140	GAAGAGCGTTGGTTGCAGG	GCCGTTCATGGTTACCACG
Group 4	Ap015	55	223	GGGGGTAACGGAGAGAGG	TGTACGAGCACGCAATC
	A043		140	GGCGTGCACAGCTTATTCC	CGAAGGTGGTTTCAGGCC
	Ac306		179	GAATATGCCGCTGCCACC	TTTCGTTGCATCCGAGCG
Group 5	Ap218	54	124	AGGGATGGAATTCTTCGATT	TTGTCACAATTCCGCTTGA
	Ap001		240	ACACGCGAACAATACAACA	ACTAATCGGCACGATGAAG
	A008		160	CGAAGGTAAGGTAAATGGAAC	GGCGGTTAAAGTTCTGG

2.5. Statistical analyses

Fragment sizing and allele calling were performed in ProSize® 2.0 (Agilent Technologies). The exported data were processed in Python/Colab, where binning and quality filtering were applied. Observed heterozygosity (H_O), expected heterozygosity (H_E), observed number of alleles per locus (N_A), effective number of alleles (N_E), Shannon's Information Index (I), number of

polymorphic loci (P), and F-statistics (Weir and Cockerham, 1984) were computed using numpy and pandas. Polymorphic information content (PIC; Botstein et al., 1980) and null allele frequencies (Brookfield, 1996) were calculated using custom functions. Nei's genetic distances (Nei, 1972) were visualized as heatmaps, and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) trees were constructed using scipy. To illustrate population differentiation, three-dimensional Linear Discriminant Analysis (LDA) plots were generated with scikit-learn and plotly. Analysis of molecular variance (AMOVA; Excoffier et al., 2007) was executed in R (poppr package) via rpy2 integration. For all randomization procedures, fixed seed values ensured reproducibility.

3. Results

Based on the calculated genetic variation statistics, the average number of observed alleles (N_A) per microsatellite locus in the Ordu population was 14.0, while the mean number of effective alleles (N_E) was 9.1188. The highest polymorphism was detected at loci Ap238 and Ap223, which produced 26 and 23 distinct alleles, respectively. Shannon's Information Index (I) values were generally high, indicating that the microsatellite markers used in this study possessed rich information content. The mean PIC value was 0.8276, demonstrating that the selected marker set had a high and reliable discriminatory power for detecting genetic differentiation among populations (Table 2).

Table 2. Summary of genetic variation and F-statistics for all loci in the Ordu population

Locus	Sample size (N)	Observed number of alleles (N_A)	Effective number of alleles (N_E)	Shannon's information index (I)	PIC
Ap223	66.0	23.0	18.6154	3.0133	0.9436
Ap238	66.0	26.0	17.0156	3.0261	0.9382
Ap273	66.0	10.0	4.0863	1.7164	0.7215
Ap243	64.0	19.0	11.4413	2.6671	0.9064
Ap085	60.0	20.0	12.2449	2.709	0.9127
Ac011	64.0	21.0	14.1241	2.814	0.9248
A113	42.0	11.0	9.8	2.3366	0.8889
At003	42.0	6.0	3.991	1.496	0.7074
A028	42.0	6.0	5.4444	1.7371	0.7899
Ap015	58.0	13.0	7.4097	2.2255	0.8516
A043	58.0	6.0	2.1399	1.1129	0.4995
Ac306	58.0	17.0	10.7134	2.554	0.8993
Ap218	56.0	10.0	6.2222	1.9992	0.8201
Ap001	76.0	15.0	9.2862	2.4273	0.8833
A008	76.0	7.0	4.2471	1.5929	0.7273
Mean	60.0	14.0	9.1188	2.2285	0.8276

Examination of heterozygosity parameters revealed that the observed heterozygosity (H_O) values exceeded the expected heterozygosity (H_E) values at several loci (Table 3). This pattern suggests a genetically diverse population, potentially influenced by heterozygote advantage, low isolation, or effective gene flow. The average expected heterozygosity (H_E), calculated according to Nei (1973), was 0.8445, whereas the observed heterozygosity (H_O) was 0.4080 (Table 3).

The Nei's (1972) genetic distance analysis performed between the Ordu population and five local *A. mellifera* populations from different regions of Türkiye revealed a clear pattern of genetic differentiation among populations (Figure 1). The highest genetic distance was observed between the Ordu and Artvin populations (D= 2.58), indicating the presence of strong microgeographic genetic divergence, despite both populations being located within the Black Sea Region.

The Ordu population also exhibited relatively high distance values from all other populations, suggesting a tendency to form a genetically distinct cluster. In contrast, the Hatay-Kırklareli (D= 0.34) and Muğla-Kırklareli (D= 0.63) pairs displayed the lowest genetic distances, implying higher gene flow or shared ancestral origins between western and southern populations (Figure 1).

Phylogenetic analysis conducted using the SplitsTree software revealed that the Ordu population was genetically distinct from the other populations, forming a separate and well-supported branch (Figure 2). This finding suggests that the Ordu population may have experienced partial isolation or undergone long-term local adaptation processes.

According to the AMOVA, 61.12% of the total genetic variation was attributed to differences among individuals. The proportion of variation among populations was 19.09%, while the variation

Table 3. Summary of heterozygosity statistics for all loci in the Ordu population

Locus	Sample size (N)	Observed heterozygosity (H_O)	Observed heterozygosity (Obs Het)	Expected homozygosity (Exp Hom)	Expected heterozygosity (H_E)	Nei's expected heterozygosity	Mean heterozygodity (Ave Het)
Ap223	66.0	0.1212	0.8788	0.0392	0.9608	0.9463	0.9463
Ap238	66.0	0.1515	0.8485	0.0443	0.9557	0.9412	0.9412
Ap273	66.0	0.9697	0.0303	0.2331	0.7669	0.7553	0.7553
Ap243	64.0	0.7188	0.2812	0.0729	0.9271	0.9126	0.9126
Ap085	60.0	0.6	0.4	0.0661	0.9339	0.9183	0.9183
Ac011	64.0	0.6562	0.3438	0.0561	0.9439	0.9292	0.9292
A113	42.0	0.7143	0.2857	0.0801	0.9199	0.8980	0.8980
At003	42.0	0.9524	0.0476	0.2323	0.7677	0.7494	0.7494
A028	42.0	1.0	0.0	0.1638	0.8362	0.8163	0.8163
Ap015	58.0	0.0345	0.9655	0.1198	0.8802	0.8650	0.8650
A043	58.0	1.0	0.0	0.4580	0.5420	0.5327	0.5327
Ac306	58.0	0.1724	0.8276	0.0774	0.9226	0.9067	0.9067
Ap218	56.0	0.5	0.5	0.1455	0.8545	0.8393	0.8393
Ap001	76.0	0.2895	0.7105	0.0958	0.9042	0.8923	0.8923
A008	76.0	1.0	0.0	0.2253	0.7747	0.7645	0.7645
Mean	60.0	0.5920	0.4080	0.1406	0.8594	0.8445	0.8445
Standard deviation		0.3595	0.3595	0.1109	0.1109	0.1095	0.1095

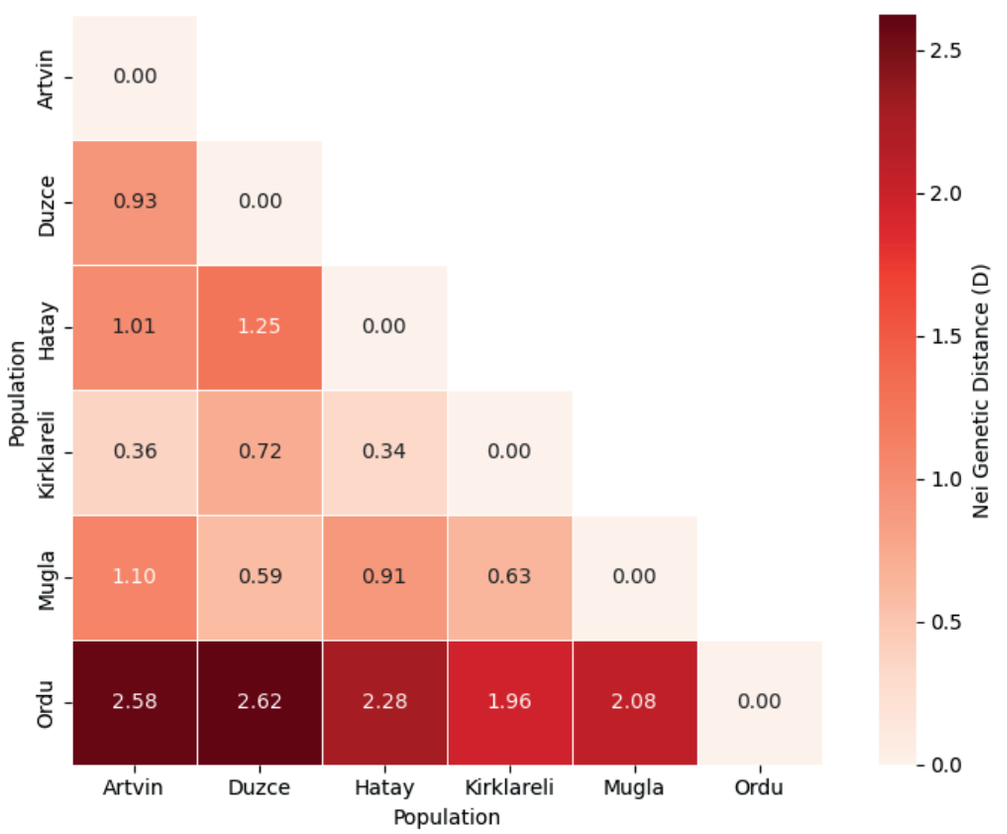

Figure 1. Heatmap of pairwise Nei's (1972) genetic distances (D) among six honey bee (*A. mellifera*) populations from Türkiye*

*: Color intensity encodes increasing D (darker= greater divergence); the diagonal is zero and only the lower triangle is displayed. The map highlights the marked divergence of the Ordu population (highest values in pairs involving Ordu, e.g., Ordu-Düzce, Ordu-Artvin) and comparatively low distances among western/southern populations (e.g., Kırklareli-Hatay)

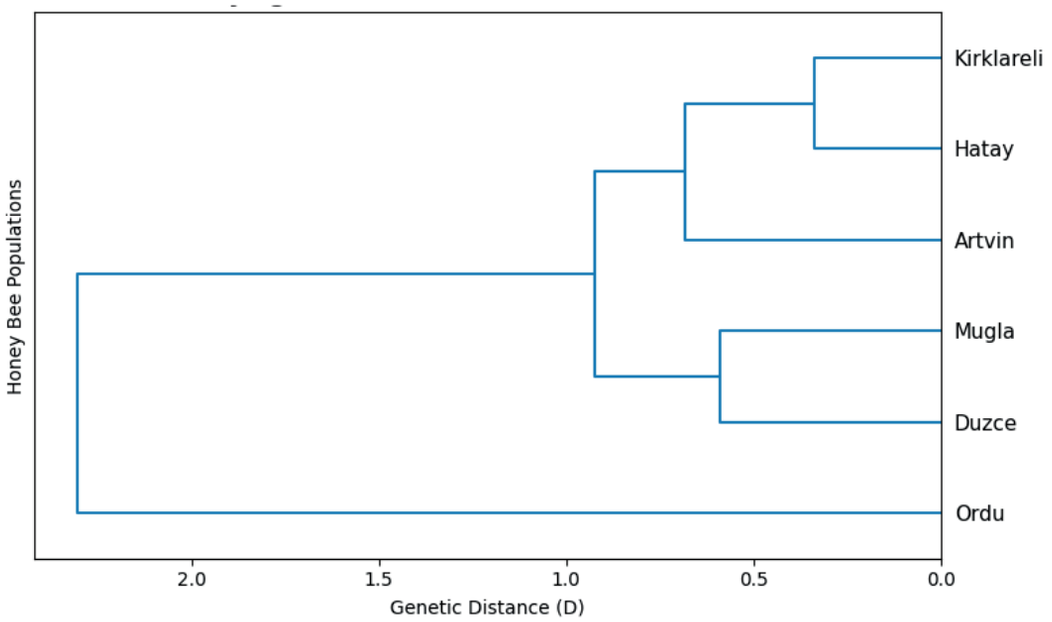

Figure 2. UPGMA phylogenetic tree based on Nei's (1972) genetic distance values among six honey bee (*A. mellifera*) populations from Türkiye*

*: The Ordu population appears as a distinct lineage, indicating pronounced genetic divergence from the other regional populations

within individuals accounted for 19.78% (Table 4). This distribution indicates that a substantial portion of the genetic diversity is concentrated within populations, although a moderate yet statistically significant differentiation also exists among populations.

The LDA-based three-dimensional ordination analysis revealed a pattern consistent with all other findings. Individuals belonging to the Ordu population were clearly separated from the other five populations within the LD1-LD3 space, forming a distinct genetic cluster (Figure 3). The

Table 4. Partitioning of genetic variation among and within populations based on AMOVA

Source of variation	Sum of squares	Variance components	Percentage of variation (%)
Among populations	633.366	1.20957	19.09256
Among individuals within populations	2432.179	3.87231	61.12306
Within individuals	349.000	1.25339	19.78438

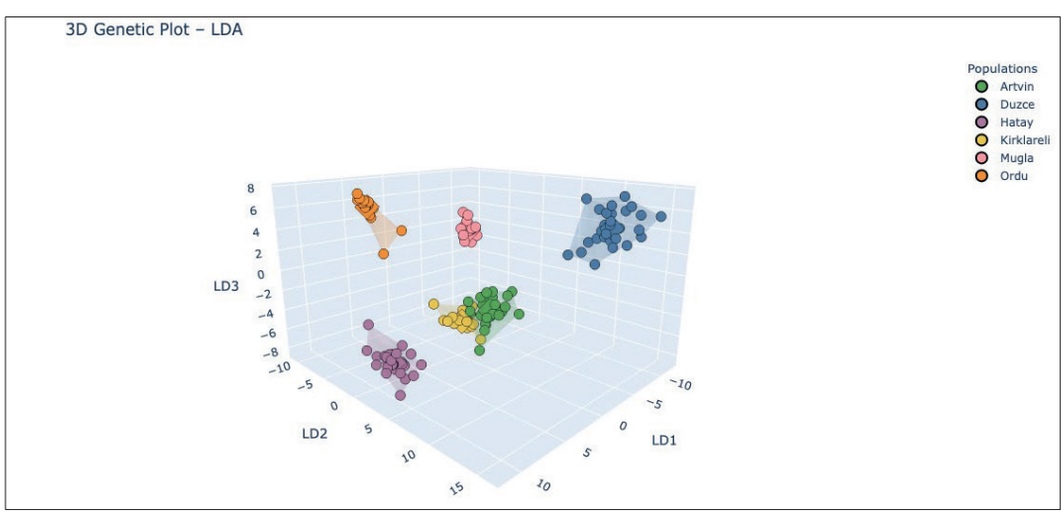

Figure 3. Three-dimensional genetic ordination (LDA)*

*: Each point represents an individual (unique fill color); population identity is indicated by marker shape and population color (used for point outlines, translucent convex hulls, and diamond centroids). Axes show the first three linear discriminants (LD1–LD3), highlighting strong separation among populations and clear clustering patterns

non-overlapping convex hull of the Ordu cluster with those of other populations supports the presence of a unique genetic structure and potential isolation of this population. In contrast, western and southern populations displayed partially overlapping distributions, indicating relatively closer genetic relationships among them.

4. Discussion and Conclusion

The honey bee (*A. mellifera*) population from Ordu Province exhibited a high level of genetic diversity based on microsatellite markers. The mean PIC values exceeded 0.5 for most loci, indicating a high degree of polymorphism and strong discriminatory power of the marker set (Çaglayan et al., 2025). Consistently, the overall expected heterozygosity (H_E = 0.8445) was high and comparable to values reported in other Turkish honey bee populations. Previous microsatellite-based studies have reported mean heterozygosity levels ranging between 54% and 68% in Anatolian bees (Bodur et al., 2007), and the HE values observed in Ordu fall within or above this range, supporting the presence of substantial allelic diversity. Although observed heterozygosity (H_O) exceeded H_E at certain loci, the overall mean H_O (0.4080) was lower than HE, indicating a general tendency toward heterozygote deficiency at the population level. This pattern may reflect colony-level structuring, non-random mating, or management-related factors influencing genotype distribution. Moreover, the presence of private alleles detected exclusively in the Ordu population further suggests that the region harbors unique genetic components within its gene pool, reinforcing its distinct genetic identity.

Nei's genetic distances and LDA visualizations demonstrated a clear genetic differentiation between Ordu and all other examined populations (pairwise D= 1.96-2.62). Notably, genetic distances remained high even with geographically close populations (e.g., Ordu-Düzce= 2.62), indicating an isolated and distinct genetic structure rather than a simple isolation-by-distance pattern. Comparable regional differentiation has been reported elsewhere; for instance, an F_ST ≈ 0.14 was found between the Western Black Sea (Yığılca) and Thrace (Kırklareli) populations (Kekeçoğlu et al., 2021). Phylogeographically, the Ordu population was clearly distinct from the Caucasian bee (*A. m. caucasica*) populations (e.g., Ordu-Artvin D= 2.58), showing closer similarity to Anatolian/Western populations. This pattern aligns with the classical biogeographic framework proposed by Ruttner (1988) and Kandemir et al. (2000), in which *A. m. anatoliaca* predominates across the Aegean, Mediterranean, and Central Anatolia regions, as well as the western and central Black Sea, whereas *A. m. caucasica* is confined mainly to Artvin and adjacent areas. Accordingly, the Ordu population appears to represent a genetically distinct subgroup within *A. m. anatoliaca*.

The observed differentiation may be shaped by geographic barriers and local selection pressures. The Black Sea region's high humidity, heavy rainfall, and diverse flora may promote morphological and behavioral adaptations among local bees. Gençer and Günbey (2020) reported that Black Sea populations can be morphologically distinguished from those in other regions, with distinct features in wing venation angles, leg lengths, and hair density (Kekeçoğlu et al., 2021). In our dataset, the high Nei distances between neighboring populations (e.g., Ordu-Düzce, Ordu-Artvin) support the hypothesis of geographic isolation and local adaptation. The province of Ordu, bounded by the sea to the north and the Eastern Black Sea Mountains to the south, likely maintained a partially isolated population historically. Such natural barriers may have limited gene flow and facilitated the preservation of local gene pools and microevolutionary divergence.

Nonetheless, modern migratory beekeeping and queen bee trade may have reduced historical isolation effects. Seasonal migratory practices across Türkiye increase interpopulation gene exchange, while the commercial distribution of highly productive subspecies such as the Caucasian bee can alter local genetic integrity. Kükrer (2013) showed that migratory beekeeping facilitates the spread of Caucasian genetic material across multiple Turkish regions. Thus, the genetic profile of the Ordu population may reflect not only natural isolation but also partial anthropogenic introgression. However, the persistently high Nei distances even with adjacent populations suggest that these human-mediated effects remain limited and spatially heterogeneous in Ordu.

Taken together, the multilocus evidence indicates that the Ordu population occupies a distinct genetic position within *A. m. anatoliaca*, reflecting regional structuring rather than random variation. Microsatellite-based comparisons and multivariate analyses consistently showed that the Ordu samples form a coherent cluster, broadly aligned with known biogeographical patterns. Previous reports describing localized differentiation in other *A. m. anatoliaca* populations, such as those from Muğla and Yığılca (Kandemir et al., 2000; Kükrer, 2013; Gençer and Günbey, 2020), also suggest the presence of regionally structured population variation within the subspecies. Further studies integrating genomic data such as single

nucleotide polymorphisms (SNPs) and mitochondrial DNA (mtDNA), together with morphometric and behavioral datasets, will be essential to validate and refine the distinctiveness of the Ordu population.

Recent genomic approaches in honey bee research have demonstrated the value of genome-wide single nucleotide polymorphisms (SNPs) and whole-genome sequencing to resolve fine-scale population structure and diversity that may be undetectable using a limited set of microsatellite loci. For example, comprehensive population genomic analyses in Western honey bees revealed concordant patterns of genetic structure and diversity across sequencing strategies, highlighting how high-resolution genomic data can clarify subspecies relationships and introgression dynamics (Chen et al., 2022). These approaches complement microsatellite studies and could be particularly informative for further resolving the genetic distinctiveness and evolutionary history of the Ordu honey bee population.

In conclusion, combined evidence from multilocus microsatellite data, including Nei's genetic distances (D= 1.96-2.62), LDA ordinations, and AMOVA results, suggests that the Ordu population may constitute a genetically distinguishable local variant within *A. m. anatoliaca*. The observed patterns are consistent with the possibility of region-specific adaptation to the environmental conditions of the Black Sea region and the long-term effects of partial geographic isolation. However, geographic isolation alone may not fully account for the distinct genetic structure observed. Contemporary apicultural practices, including localized breeding strategies, regulated queen exchange, and the management of migratory beekeeping activities, may also have contributed to shaping the current genetic profile of the Ordu population. Accordingly, this population may be considered a regionally structured genetic unit within *A. m. anatoliaca*, whose genetic integrity appears to reflect the combined influence of natural and anthropogenic factors. The sustainable management of this genetic resource should therefore involve strengthening regional breeding nuclei, carefully regulating queen exchange, monitoring migratory beekeeping practices, and establishing standardized genomic and morphometric monitoring frameworks. Such measures would support the conservation and informed utilization of this locally differentiated honey bee population, while aligning with Türkiye's long-term goals for resilient and productive apiculture.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Conceptualization, Methodology, Formal Analysis, Visualization, Software, Writing-Original Draft Preparation, *B.İ. YILDIZ*; Material, Investigation, Writing-Review & Editing, *M. AKGÜN*; Material, Investigation, Writing-Review & Editing, *Z. SÖNMEZ*; Material, Investigation, Writing-Review & Editing, *A. ÇİFTÇİ*; Material, Investigation, Writing-Review & Editing, *E. SOYDAN*; Conceptualization, Methodology, Formal Analysis, Visualization, Supervision, Project Administration, Funding Acquisition, Writing-Original Draft Preparation, *K. KARABAĞ*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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