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Microsatellite-based genetic characterisation of honeybee populations from Bingöl

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ABSTRACT

The conservation of endemic genetic resources is crucial for maintaining genetic diversity in animal production, particularly in honeybees, where preserving local adaptations is challenging. Türkiye's rich flora, supported by diverse climates, fosters various honeybee races and ecotypes. Bingöl province, situated at the intersection of three phytogeographic regions, is a biodiversity hotspot and a key beekeeping center. Genetic characterization of 141 worker bees from five Bingöl locations, using 30 microsatellite loci, revealed 6.77% genetic variation among locations, 65.69% among individuals, and 27.54% overall. The population exhibited signs of a genetic bottleneck and deviated from Hardy-Weinberg equilibrium. Phylogenetic analysis distinguished the Bingöl honeybee population from other Turkish populations, representing five different honeybee genotypes: Muğla (*Apis mellifera anatolica*), Hatay (*Apis mellifera syriaca*), Kırklareli (*Apis mellifera carnica*), Artvin (*Apis mellifera caucasica*), and Düzce (*Apis mellifera anatolica*, Yığılca ecotype), revealing 18 specific alleles that may indicate local genetic distinctiveness.

1. Introduction

Honeybees (*Apis mellifera*) are distributed across diverse ecological conditions, leading to the development of various morphological, physiological, and behavioral traits (Ruttner 1988; Rinderer et al. 2010). These bees are generally categorized into six evolutionary lineages and over 25 races, each shaped by their geographic and environmental contexts (Ruttner 1988). In Türkiye's Anatolian region, five distinct races (*A.m. anatoliaca, A.m. caucasica, A.m. meda, A.m. syriaca, A.m. carnica*) have been identified, representing roughly 20% of the world's honeybee genetic diversity (Karabağ et al. 2020). Türkiye's geographical diversity has contributed to the development of endemic genotypes yet to be fully identified.

Bingöl province, located at the junction of three phytogeographical regions in eastern Türkiye, stands out with its unique floral diversity and serves as a significant beekeeping center (Kesik et al. 2022). The local Bingöl honeybee population is known for its distinctive traits, and literature suggests superior performance compared to other breeds (Şahin and Esim 2024). However, the genetic structure of this population has not been fully elucidated. Earlier studies have shown the presence of *A.m. meda* in neighboring regions, including Southeast Anatolia, with populations found near the Iranian border (Ruttner 1988, Özdil et al. 2012; Kambur Acar and Kekeçoğlu 2020).

Microsatellites, or simple sequence repeats (SSRs), are prominent genetic markers extensively used in population genetics, conservation, and evolutionary biology due to their high mutation rates and Mendelian inheritance (Abdelkrim et al. 2009). Their high polymorphism makes them effective tools for studying population structures and assessing genetic distances (Chen et al. 2004; Gomes et al. 2013). Even in the genomic era, microsatellites remain competitive due to their specific advantages in diversity studies (Mukherjee et al. 2022). As such, microsatellites have been extensively used to study honeybee diversity, also in Türkiye (Alburaki et al. 2013; Karabağ et al. 2020; Yıldız et al. 2023).

This study focuses on the genetic characterization of the honeybee population in Bingöl, examining its genetic diversity and potential differentiation within Turkish honeybee genotypes using 30 microsatellite markers.

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2. Materials and Methods

2.1. Honeybee sampling

A total of 141 worker bees of similar physiological age were collected from 141 colonies in five locations (Ağaçeli, Gökçeli, Alatepe-1, Alatepe-2, and Yenibaşlar) in Bingöl Province (Figure 1). These colonies were selected based on observations of experienced beekeepers to represent the local honeybee population. Bingöl Province is located between 41°20' and 39°56' eastern longitudes, and 39°31' and 36°28' northern latitudes, with the city center situated at an altitude of 1151 meters. Sampling was conducted with care to avoid colonies associated with migratory beekeeping practices, ensuring the collected bees were representative of the native population. The worker bees, selected based on their similar appearance, were stored in 50 cc Falcon tubes containing alcohol and kept at -20°C. Molecular analyses were subsequently performed at the Akdeniz University Animal Biotechnology Laboratory in 2023.

2.2. DNA extraction and microsatellite genotyping

For DNA extraction, one worker bee per colony was selected. After removal from alcohol, the bees were rinsed in sterile water, dried, and the thoracic sections were isolated. Samples were ground in liquid nitrogen, and DNA was extracted using the Bio Basic DNA extraction kit. DNA quality and quantity were assessed via agarose gel electrophoresis and a Biodrop device, then stored at -20°C. Genomic variations were estimated using 30 microsatellite loci from the literature (Solignac et al. 2003). PCR reactions were conducted using 2 µl of 50 ng/µl DNA, with a total volume of 25 µl. PCR cycling included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and extension. PCR products were analyzed on a 2% agarose gel, and fragment lengths were measured using an AATI fragment analyzer.

2.3. Statistical analysis

Microsatellite fragment lengths were scored using PROSize v.2.0 software, and various genetic parameters, such as the number of loci (N), polymorphic loci (NP), observed alleles (NA), effective alleles (NE), expected heterozygosity (HE), observed heterozygosity (HO), inbreeding coefficient (FIS), and Hardy-Weinberg equilibrium (HWE) were calculated using Popgene v.1.32 (Yeh et al. 1997). Polymorphic Information Content (PIC) was estimated using microsatellite toolkit software (Park 2001), and null alleles were assessed with ML-NullFreq (Kalinowski and Taper 2006). AMOVA was performed using Arlequin v.3.11 (Excoffier et al. 2007). Bottleneck v.1.2 (Cornuet and Luikart 1996) was used to detect genetic bottlenecks via the Two-Phase Mutation Model (TPM). Sign tests and Wilcoxon signed rank tests were applied to assess deviation from HWE and bottleneck signatures. Allele frequency distribution was plotted according to the method of Luikart et al. (1998). Bayesian clustering analysis of genetic admixture was conducted using Structure v.2.3.3 (Pritchard et al. 2000), and FCA was performed with Genetix v.4.05 (Belkhir et al. 1996). The optimal number of genetic clusters (K) was determined using ΔK statistics via Structure Harvester (Earl and von Hold 2012) and the Evanno method (Evanno et al. 2005). To understand the phylogenetic relationships and differences between the Bingöl honeybee population and other Turkish genotypes, microsatellite data from five different honeybee populations reported by Karabağ et al. (2020) were utilized, including 30 loci. These populations represent distinct genotypes: Muğla (*A. mellifera anatolica*), Hatay (*A. mellifera syriaca*), Kırklareli (*A. mellifera carnica*), Artvin (*A. mellifera caucasica*), and Düzce (*A. mellifera anatolica*, Yığılca ecotype).

3. Results and Discussion

Genetic parameters of the Bingöl honeybee population are shown in Figure 2. Analysis revealed an average of 18 observed alleles (N_A), higher than previous studies (Bodur et al. 2007; Ivgin Tunca 2009; Karabağ et al. 2020; Yıldız et al. 2023), suggesting adequate sample size (Mielnik-Sikorska et al. 2013). The average number of effective alleles (N_E) was 9.23, ranging from 19.62 at locus Ap223 to 2.18 at Ac011. Loci with high N_E values (e.g., Ap223, A113, Ap249) highlight genetic variation in the population. The average Polymorphic Information Content (PIC) was 0.84, with the highest (0.95) at Ap2523 and the lowest (0.52) at Ac011, indicating the suitability of these loci for genetic diversity studies (Botstein et al. 1980), consistent with previous findings in Türkiye (Karabağ et al. 2020; Yıldız et al. 2023).

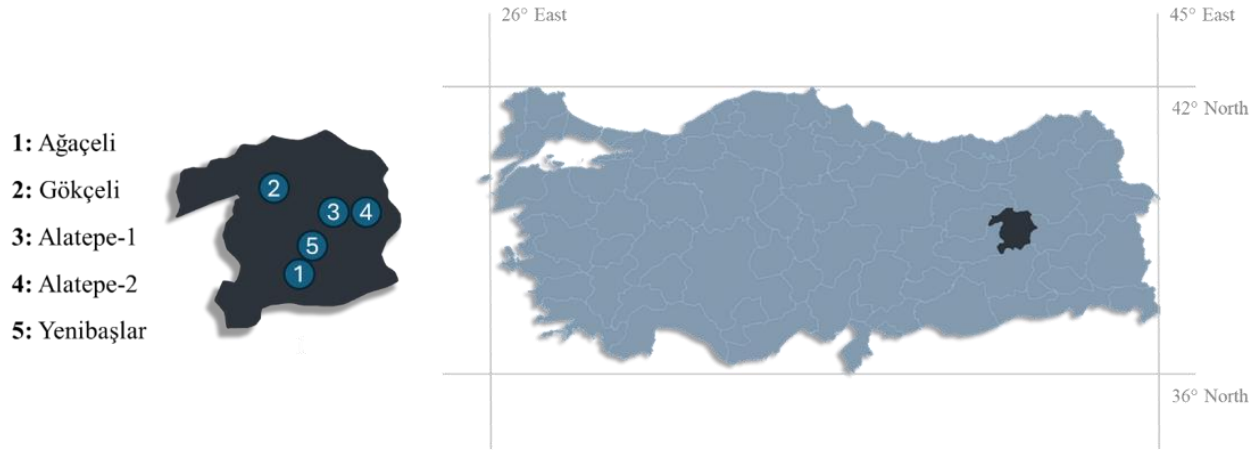


Figure 1. Sampling locations in the Bingöl Province.

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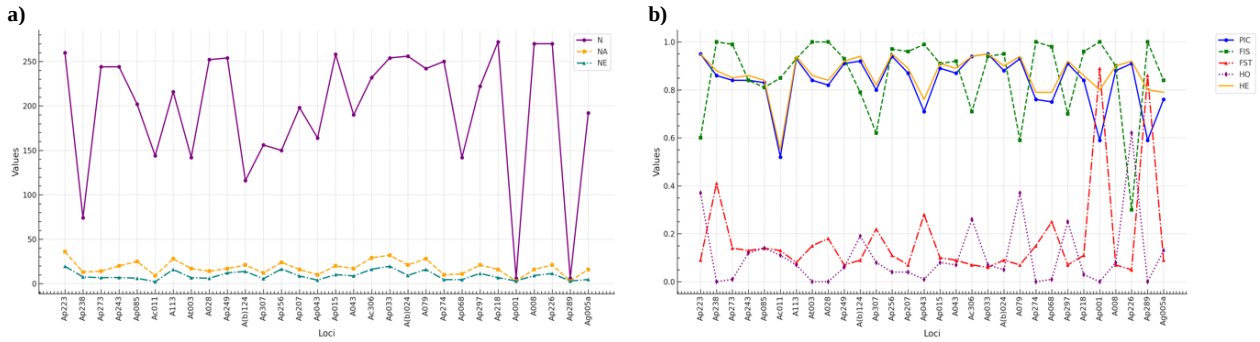


Figure 2. Genetic diversity profile of Bingöl honeybee populations using 30 microsatellite loci. **a)** (N): number of total alleles used in each locus; (N_A): number of observed alleles; (N_E): number of effective alleles. **b)** (PIC): polymorphic information content; (F_{IS}): inbreeding coefficient; (F_{ST}): genetic differentiation coefficient; (H_O): observed heterozygosity; (H_E): expected heterozygosity.

The Bingöl honeybee populations show a positive inbreeding coefficient (F_{IS}), with an average of 0.85, indicating higher homozygosity, ranging from 0.30 (Ap226) to 1 (Ap238, At003, A028, Ap274, Ap001, Ap289). F_{IS} values above 0.40 suggest significant homozygosity, often seen in small, isolated populations (Simon and Buchenauer 1993; Castric et al. 2002). Genetic differentiation (FST) was 0.07, reflecting increased relatedness within the populations. Observed heterozygosity (H_O) averaged 0.11, significantly lower than expected heterozygosity (H_E) of 0.86, indicating a predominance of homozygous individuals. Compared to other studies, H_O in this study is lower, with Franck et al. (2000) reporting a range of 0.367-0.872 in Lebanese populations, and Bodur et al. (2007) reporting H_O between 0.52-0.67 across 12 Turkish populations. Similarly, other studies (Ivgin Tunca 2009; Karabağ et al. 2020; Yıldız et al. 2023) show higher heterozygosity levels in various honeybee populations.

The values across the five locations were similar. Ağaçeli and Alatepe-1 had the highest PIC values (0.70), while Yenibaşlar had the highest FIS (0.96), N_A (9.24), and N_E (5.85). Ağaçeli had the highest expected heterozygosity (H_E) at 0.81, and Alatepe-1 showed the highest observed heterozygosity (H_O) at 0.15 (Figure 3).

Genetic diversity among the five populations was evaluated using Arlequin, calculating binary F_{ST} values (Weir and Cockerham 1984). Ağaçeli and Alatepe-1 were the closest populations (-0.001), while Alatepe-1 and Yenibaşlar were the most distant (0.16). Genetic distances, calculated using Nei's method (Nei 1972), ranged from 1.532 (Yenibaşlar and Gökçeli) to 0.026 (Yenibaşlar and Alatepe-2) (Figure 4).

AMOVA analysis revealed that 6.77% of genetic variation was between populations, 65.69% among individuals, and 27.54% overall. Significant genetic differences were confirmed (F_{IT}: 0.73, F_{ST}: 0.07, F_{IS}: 0.71, P<0.05), suggesting low variation between locations, indicating a shared bee genotype. Bayesian cluster analysis (Structure software) showed three genetic groups (K= 3), with Yenibaşlar being distinct. Genetic differentiation between Alatepe-2 and Yenibaşlar may be due to the use of common queen bee genotypes and geographic isolation (Figure 5).

Bottleneck analyses of Bingöl bee populations used the Two-Phase Mutation (TPM) model, which is suitable for microsatellite loci (Luikart et al. 1998; Piry et al. 1999; Karabağ et al. 2020; Yıldız et al. 2023). This model provides insights into historical population size changes, crucial for understanding genetic diversity. The standardized differences test indicated a significant bottleneck effect in all populations (P<0.05), suggesting reduced genetic variation likely due to environmental or anthropogenic factors (Table 1).

Significant deviations from Hardy-Weinberg equilibrium (P<0.01) were observed, indicating bottlenecks common in small, isolated populations (Luenser et al. 2005; Mukherjee et al. 2022) The limited variation in the Bingöl bee populations may be due to the frequent use of queen bees from the same source, a known issue in Turkish beekeeping (Yıldız et al. 2023). Introducing queens from diverse sources can increase genetic diversity by adding new alleles to the local gene pool through drones (Bourgeois et al. 2008). Similar bottlenecks have been observed in honeybee populations in the Balearic Islands, with low Varroa destructor infestations (De La Rua et al. 2003).

To determine the race of the Bingöl honeybee population, it was compared to populations from Muğla (*A. mellifera anatolica*), Hatay (*A. mellifera syriaca*), Kırklareli (*A. mellifera carnica*), Artvin (*A. mellifera caucasica*), and Düzce (*A. mellifera anatolica*) using the same microsatellite loci (Karabağ et al. 2020). Structure analysis (ΔK= 5) and FCA revealed that the Bingöl population is clearly distinct from the other five populations (Figure 6).

This study identified 18 specific alleles (e.g., ap238, ac306, ap223) unique to the Bingöl honeybee population. The presence of these specific alleles suggests limited gene flow and a unique genetic structure for the Bingöl population. Previous studies (Kence et al. 1997; Kandemir et al. 2000) reported the presence of *A. mellifera meda* hybrids in the Bingöl region, with rare alleles for enzymes such as Pgm and Est. While these findings align with earlier morphometric and genetic studies (Ruttner 1988; Kandemir et al. 2000; Özdil et al. 2012), suggesting a possible connection to *A. mellifera meda*, further studies are required to confirm whether the Bingöl population represents hybrids or a distinct ecotype influenced by *A. mellifera meda*.

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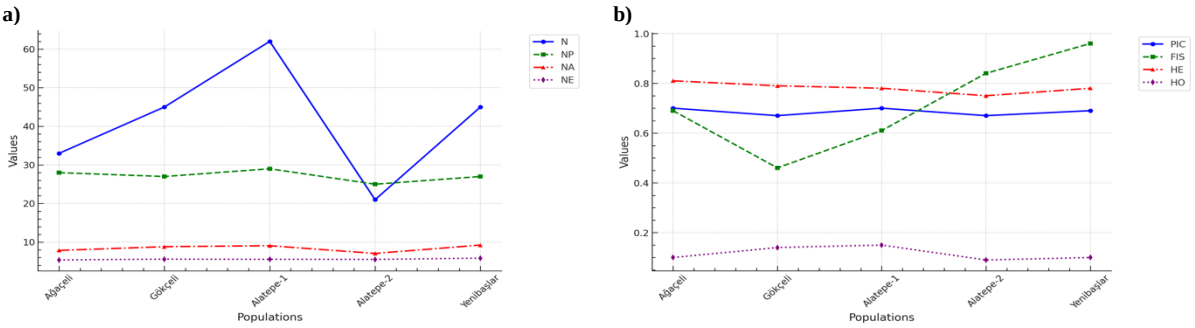


Figure 3. Genetic variation values of honeybee colonies across five collection locations in Bingöl. **a)** N: total alleles used in each locus; (N_P): number of polymorphic loci; (N_A): number of observed alleles; (N_E): number of effective alleles. **b)** (PIC): polymorphic information content; (F_{IS}): inbreeding coefficient, (H_O): observed heterozygosity; (H_E): expected heterozygosity.

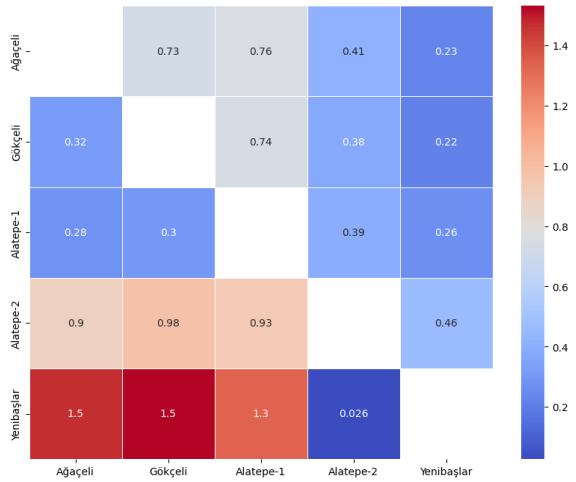


Figure 4. Genetic differences of of honeybee colonies across five collection locations in Bingöl. Genetic identity data are shown above the diagonal, while genetic distance data are shown below it (Nei 1972).

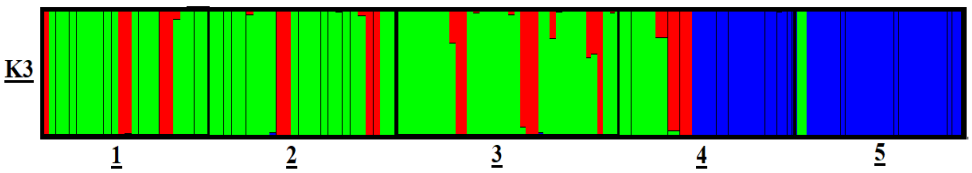


Figure 5. Genetic structure analysis of five honeybee populations in theBingöl province. 1: Ağaçeli, 2: Gökçeli, 3: Alatepe-1, 4: Alatepe-2, 5: Yenibaşlar. Each color corresponds to a genetic cluster, and the length of the colored segment represents the membership coefficient of the individual in the cluster according to cluster analysis.

Table 1. The bottleneck analysis utilizing the standardized differences test under the two-phase mutation model

Statistical Tests		TPM				
		Ağaçeli	**Gökçeli**	**Alatepe-1**	**Alatepe-2**	**Yenibaşlar**
Sign test	EHE	16.60	16.10	17.14	14.70	15.06
	HD	4.00	7.00	6.00	2.00	7.00
	HE	24.00	20.00	23.00	23.00	19.00
	P	0.00	0.09	0.02	0.00	0.08
Standardized differences test	T2	3.38	1.69	2.47	4.34	2.43
	P	0.00	0.05	0.01	0.00	0.01
Wilcoxon's signed rank test	HD (P)	1.00	0.98	1.00	1.00	1.00
	HE (P)	0.00	0.02	0.00	0.00	0.00
	HDE (P)	0.00	0.04	0.00	0.00	0.01

TPM, two-phased model; EHE, expected heterozygosity excess; HD, heterozygosity deficiency; HE, heterozygosity excess; HDE, heterozygosity excess or deficiency; T2, standardized differences test. Positive values of the bottleneck statistic T2 indicate an excess of gene diversity due to a recent reduction in effective population size, while negative values suggest recent population expansion without immigration or immigration of private alleles.

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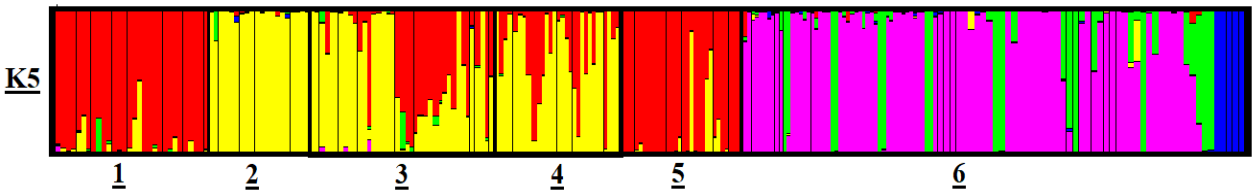


Figure 6. Genetic structure analysis of six honeybee populations in Türkiye. 1: Muğla (*A. mellifera anatolica*), 2: Hatay (*A. mellifera syriaca*), 3: Kırklareli (*A. mellifera carnica*), 4: Artvin (*A. mellifera caucasica*), 5: Düzce (*A. mellifera anatolica*) 6: Bingöl. Each color corresponds to a genetic cluster, and the length of the colored segment represents the membership coefficient of the individual in the cluster according to cluster analysis.

4. Conclusions

Our study on Bingöl honeybee populations revealed genetic bottlenecks, indicating historical reductions in population size due to environmental or human factors. The allele frequency distribution and identification of 18 specific alleles suggest a unique genetic profile, which might reflect local adaptation or potential hybridization with A. mellifera meda. While this raises the possibility of Bingöl honeybees being a region-specific ecotype, further studies are required to substantiate this classification. The findings underscore the importance of genetic management in beekeeping practices, including the introduction of diverse queen bees to maintain genetic diversity and prevent inbreeding. Future research should focus on monitoring genetic changes and population dynamics over time.

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