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Original article (Orijinal araştırma)

A study of whether the genetic variation decreased or not in the protected Caucasian bee, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae) population in isolated regions[1]

İzole bölgelerde korunan Kafkas arısı, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae) popülasyonunda genetik varyasyonun azalıp azalmadığına dair bir çalışma

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Abstract

The Caucasian honeybee, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae), is one of the most productive bee subspecies. This subspecies, which has special importance for Türkiye, has been taken under protection in two isolated regions (Artvin and Ardahan) since 2000. To date, no study has been conducted on whether genetic diversity has decreased in these protected Caucasian honeybee colonies. Therefore in 2022, worker bees were collected from 100 different colonies in 15 different locations in these two regions and their genetic variations were examined using 30 microsatellite loci. The average number of alleles per locus was 13.57, and the loci had a high level of information content according to the PIC (0.7) value. F_{IS} (0.96) and F_{ST} (0.01) values showed low genetic diversity and high inbreeding in populations. Genetic variations were calculated as 0.77% among populations, 99.23% among individuals in populations, and 0% among all individuals. Also, populations deviated from the Hardy-Weinberg equilibrium ($p<0.001$). Significant bottleneck evidence was found for Artvin in the analysis results using the two-phase mutation model. These results provide important information that can be used as a guide for Caucasian bee breeding strategies and conservation programs.

Keywords: *Apis mellifera*, Caucasian bee, genetic variation, microsatellite, population structure

Öz

Kafkas arısı, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae), en verimli arı alt türlerinden biridir. Türkiye için özel bir öneme sahip olan bu alt tür, 2000 yılından itibaren iki izole bölgede (Artvin ve Ardahan) koruma altına alınmıştır. Bugüne kadar korunan Kafkas bal arısı kolonilerinde genetik çeşitliliğin azalıp azalmadığına dair bir çalışmaya rastlanmamıştır. Bu nedenle 2022 yılında, bu iki izole bölgede 15 farklı lokasyonda bulunan 100 farklı koloniden işçi arılar toplanmış ve 30 mikrosatelit lokusu kullanılarak genetik varyasyonlar incelenmiştir. Lokus başına ortalama allel sayısı 13.57 bulunmuştur ve PIC (0.7) değerine göre lokuslar yüksek düzeyde bilgi içeriğine sahiptir. F_{IS} (0.96) ve F_{ST} (0.01) değerleri popülasyonlarda düşük genetik çeşitlilik ve yüksek akrabalı yetiştirme olduğunu göstermiştir. Genetik varyasyonlar, popülasyonlar arasında %0.77, popülasyonlardaki bireyler arasında %99.23 ve tüm bireyler arasında %0 olarak hesaplanmıştır. Ayrıca, popülasyonlar Hardy-Weinberg dengesinden sapmıştır ($P<0.001$). İki fazlı mutasyon modeli kullanılarak yapılan analiz sonuçlarında Artvin için önemli bir darboğaz kanıtı bulunmuştur. Bu sonuçlar, Kafkas arısı ıslah stratejileri ve koruma programları için kılavuz olarak kullanılabilecek önemli bilgiler sağlamaktadır.

Anahtar sözcükler: *Apis mellifera*, kafkas arısı, genetik varyasyon, mikrosatelit, popülasyon yapısı

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Introduction

Apis mellifera, naturally spreads over a wide geographic range including Europe, Africa, West and Central Asia. Low migration capability and limited population size have resulted in presence of approximately 30 subspecies and high levels of genetic variation in this large geography (Ruttner, 1988). Within these subspecies, there are also ecotypes and breeding lines that are very important for researchers and beekeepers (Oleksa & Tofilski, 2015). Interactions between subspecies often occur through human activities. Commercially managed bee colonies are transported between remote areas for pollination services and food access. These activities affect genetic variability (Bouga et al., 2011). Although various revisions have been made in honey bees classification; genetic, morphometric and ethological studies have identified four major evolutionary lineages in the honey bee: African ancestry (A), Western and Northern European ancestry (M), Southeast European ancestry (C), and Middle Eastern ancestry (O) (Ruttner, 1988; Arias et al., 2006; Bouga et al., 2011; Nawrocka et al., 2018).

Many molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and mitochondrial DNA (mtDNA) analyses have been used in honey bee population genetics studies, but for the last 20 years, microsatellite loci have been quite widely used due to their features such as high polymorphism, multiallelicity, abundance in the genome and easy scorable (Kandemir & Kence, 1995; Smith et al., 1997; Bodur et al., 2007; Kekeçoğlu et al., 2009; Özdil et al., 2009; Liu et al., 2016; Rahimi et al., 2016; Haddad et al., 2018; Hassett et al., 2018; Yu et al., 2019; Özdil et al., 2022). Latterly, single nucleotide polymorphisms (SNPs) have been used in population genetics studies, however, thanks to the advantages provided by microsatellites, they could not get ahead of microsatellites even in the genomic era (Zimmerman et al., 2020; Mukherjee et al., 2022; Wang et al., 2022). Genetic diversity and species richness in honey bee populations in Türkiye have been demonstrated by microsatellite and mtDNA studies (Kandemir et al., 2006; Bodur et al., 2007; Ivgin Tunca, 2009; Özdil et al., 2009). This extensive research has revealed the presence of five subspecies of honey bees; Anatolian bee (*Apis mellifera anatoliaca* Maa, 1953), Caucasian bee, *Apis mellifera caucasica* Pollmann, 1889, Iranian bee, *Apis mellifera meda* Skorikov, 1929, Syrian bee, *Apis mellifera syriaca* Skorikov, 1829) and Carniolan bee, *Apis mellifera carnica* Pollmann, 1879 (Hymenoptera: Apidae) (Kandemir et al., 2000), and it has about 20% of the honey bee subspecies in the world. Anatolia is a gene center for honey bees (Ruttner, 1988) and provides a great diversity in almost every characteristic of honey bees. Although many breeding programs have been implemented around the world, genetic variation has remained insufficient in the production of resistant stocks (Rinderer et al., 2010). At this point, this genetic diversity provides an advantage in possible breeding studies in our country.

Caucasian honey bee, which is one of the important gene resources in Anatolia, is the most preferred subspecies due to its superior characteristics in terms of honey production, its calm and docile behavior (Ruttner, 1988). The natural range of Caucasian honey bee has been extended by humans from the Caucasus to Bulgaria (Ivanova et al., 2007) along with a large number of hives brought to Ukraine, Germany and France (Ruttner, 1988). In Türkiye, it is located in the northeastern region, in neighboring provinces, especially in Ardahan, Artvin and Kars, but after the migratory beekeeping that started in the 1950s, the number of pure bee colonies in this region has gradually decreased (Kırpık et al., 2010). Likewise, Fıratlı and Budak (1994) reported that the genetic structure of honey bee populations in Türkiye have changed and confusion has increased in the populations. With the studies carried out by the Ministry of Agriculture and Forestry in 2000, Artvin and Ardahan provinces were declared as isolated regions for the *A.m caucasica* subspecies and tried to be taken under protection (Gül & Nergiz, 2022). The results of the studies conducted after the declaration have shown that there are still concerns about genetic diversity.

Understanding the genetic diversity that remains in natural populations is known to be crucial for conservation plans (Kuo & Jansen, 2004). Although the determination of genetic diversity in the Caucasian

honey bee colonies, which have been under protection in two isolated regions for 20 years, is of great importance, there is not enough information about the situation in literature. Therefore, we aimed to detect the genetic variations present in the Caucasian honey bee colonies in this study.

Materials and Methods

Sample collection

As material, a total of 100 worker bees were collected from 100 different colonies in 15 locations in two provinces (Artvin and Ardahan) in 2022 (Table 1).

Table 1. Geographical coordinates and altitudes of the colonies where bee samples were taken

	Locations	Coordinate	Altitude (m)
Artvin	City center	41º10′N 41º49′E	524
	Ardanuç	41º07′N 42º03′E	486
	Arhavi	41º21′N 41º18′E	7
	Borçka	41º21′N 41º40′E	124
	Hopa	41º23′N 41º25′E	8
	Kemalpaşa	41º28′N 41º31′E	15
	Murgul	41º15′N 41º39′E	952
	Şavşat	41º15′N 42º21′E	1108
	Yusufeli	40º49′N 41º32′E	601
Ardahan	City Center	41º06′N 42º42′E	1807
	Çıldır	41º07′N 43º07′E	1975
	Damal	41º20′N 42º50′E	2051
	Göle	40º47′N 42º36′E	2018
	Hanak	41º14′N 42º50′E	1818
	Posof	41º30′N 42º43′E	1545

DNA extraction and microsatellite amplification

Before DNA isolation, the samples were arranged on blotting paper one by one and kept at room temperature for 3 hours to remove the alcohol. The head and thorax of the bees were separated from the other body parts with the help of sterile tweezers and placed in numbered 1.5 ml microcentrifuge tubes. Liquid nitrogen was added to each microcentrifuge tube and the samples were homogenized with the help of sterile plastic rods. Total DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). The quality and quantity of the DNAs, which were kept at +4°C overnight, were determined by BioDrop spectrophotometer. In addition, the quality of each individual's genomic DNA was verified by performing electrophoresis on a 1% agarose gel. DNA samples were stored at -20°C until used in the next step.

For the genetic characterization of the Caucasian bee, 30 microsatellite markers proposed by Solignac et al. (2003) were used. PCR amplifications were performed using Xpert Fast Hotstart Mastermix (Grisp, Porto, Portugal) with 2 µl of template DNA (50 ng/µl) in a total mixture volume of 25 µl. The thermal cycler program was 94°C for an initial denaturation for 5 min; 35 cycles of 94°C for 30 s, 30 s at the primer-specific annealing temperature, and 72°C for 45 s; and a final 72°C for 5 min. PCR products were electrophoresed on a 2% agarose gel in TBE buffer (25 mM Tris, 25 mM Boric acid, 50 mM EDTA, pH 8.0). Precise fragment lengths of the PCR products were determined on the AATI fragment analyzer.

Statistical analysis

The fragment lengths were manually scored with the PROSize2.0 software (Advanced Analytic Technologies Inc., Ankeny, IA, USA). Belonging to populations; number of loci (N), number of polymorphic loci (N_P), number of observed alleles (N_A), number of effective alleles (N_E), expected (H_E) and observed (H_O) heterozygous values, F-statistics and Hardy-Weinberg equilibrium (HWE) were calculated using Popgene v.1.32 software (Yeh et al., 1997). Microsatellite toolkit software was used to calculate the polymorphic information content (PIC) of microsatellite markers (Park, 2001). Null alleles, also known as non-amplified alleles, were predicted using ML-NullFreq software (Kalinowski & Taper, 2006). Molecular variance (AMOVA) (Excoffier et al., 1992) was calculated with Arlequin v.3.11 software (Excoffier et al., 2007). The bottleneck hypothesis was investigated using Bottleneck 1.2.02 software (Cornuet & Luikart, 1996). Due to the number of loci (>20) and samples; Two-phase Mutation Model (TPM), sign test (to calculate how many loci with heterozygosity deficiency or heterozygosity excess), Wilcoxon's signed rank test (to determine whether heterozygosity deficiency or excess), standardized differences test (for the genetic signature of bottlenecks in the honey bee populations studied) were used to determine the bottleneck.

The genetic structure and genetic admixture levels of the populations were estimated with the Structure v.2.3.3 software (Pritchard et al., 2000) using the Bayesian clustering algorithm. Factorial correspondence analysis (FCA) was performed using Genetix v.4.05 software (Belkhir et al., 1996-2004) to reveal relationships between individuals in populations and to examine genotypic data in a three-dimensional plane.

Results and Discussion

The various genetic parameters tested for the Caucasian honey bee populations are described in Table 2. When all microsatellite loci were examined, the mean N_A was calculated as 13.57. It is seen that this value is higher than previous studies (Bodur et al., 2007; Ivgin Tunca, 2009; Karabağ et al., 2020). The high N_A value indicates that the number of samples used in the study is sufficient to measure genetic diversity (Mielnik-Sikorska et al., 2013). While the mean N_E was 4.91, the highest and lowest N_E values were calculated as 13.84 (Ap256) and 1.78 (A028). Accordingly, it can be suggested that loci above this mean N_E value (Ap223, Ap238, A113, A(b)124, Ap256, Ac306, Ap033, Ap068, Ap001, Ap289) should be used in genetic characterization studies in honey bees. While the mean PIC value for all loci was found 0.71, the highest and lowest PIC values were found as 0.92 (Ap256) and 0.39 (Ag005a). PIC values were on average above 0.5, meaning that the selected loci had high information content and were suitable for genetic diversity study (Botstein et al., 1980).

The mean F_{IS} value for all loci was found as 0.96. Also, the lowest F_{IS} value was 0.39 (Ap001) and except for the Ap223, Ap238, Ap085, Ap001 loci, the F_{IS} value was found to be 1 in all loci. The F_{IS} value is used to determine the deviation from the Hardy-Weinberg equilibrium in a population and is one of the important indicators in determining conservation priorities for populations. Positive values obtained indicate a deficiency of heterozygosity (Bodur, 2005). According to Simon & Buchenauer (1993), homozygosity reaches a dangerous level when the F_{IS} value is greater than 0.40. Inbreeding is the major cause of heterozygous deficiency in isolated and relatively small areas (Castric et al., 2002). The fact that the genetic difference was found to be 0.01 in terms of F_{ST} also supports the increasing inbreeding in the Artvin and Ardahan isolated regions.

Table 2. Descriptive statistics for genetic diversity of all population over 30 microsatellite loci

Locus	N	N_A	N_E	PIC	F_{IS}	F_{ST}	H_O	H_E	Mean H
Ap223	170.00	24.00	8.98	0.88	0.97	0.01	0.02	0.89	0.88
Ap238	164.00	23.00	6.69	0.84	0.78	0.01	0.18	0.86	0.84
Ap273	172.00	13.00	4.15	0.74	1.00	0.02	0.00	0.76	0.74
Ap243	186.00	14.00	3.58	0.69	1.00	0.01	0.00	0.72	0.71
Ap085	186.00	16.00	3.18	0.65	0.90	0.03	0.06	0.69	0.66
Ac011	184.00	12.00	4.05	0.72	1.00	0.04	0.00	0.76	0.72
A113	176.00	16.00	7.55	0.85	1.00	0.01	0.00	0.87	0.86
At003	176.00	9.00	2.54	0.58	1.00	0.01	0.00	0.61	0.60
A028	176.00	9.00	2.02	0.48	1.00	0.00	0.00	0.51	0.50
Ap249	176.00	11.00	4.10	0.73	1.00	0.00	0.00	0.76	0.75
A(b)124	176.00	17.00	6.34	0.82	1.00	0.01	0.00	0.85	0.84
Ap307	176.00	5.00	1.87	0.41	1.00	0.04	0.00	0.47	0.44
Ap256	192.00	24.00	13.84	0.92	1.00	0.02	0.00	0.93	0.91
Ap207	194.00	10.00	4.09	0.73	1.00	0.02	0.00	0.76	0.74
Ap043	194.00	7.00	2.21	0.50	1.00	0.01	0.00	0.55	0.54
Ap015	188.00	12.00	4.14	0.73	1.00	0.00	0.00	0.76	0.75
A043	192.00	10.00	4.69	0.76	1.00	0.01	0.00	0.79	0.78
Ac306	190.00	18.00	8.11	0.86	1.00	0.02	0.00	0.88	0.86
Ap033	168.00	20.00	6.73	0.82	1.00	0.02	0.00	0.86	0.83
A(b)024	168.00	16.00	4.91	0.78	1.00	0.02	0.00	0.80	0.78
A079	188.00	13.00	4.62	0.75	1.00	0.01	0.00	0.79	0.77
Ap274	180.00	14.00	3.03	0.63	1.00	0.01	0.00	0.67	0.66
Ap068	176.00	16.00	7.08	0.84	1.00	0.01	0.00	0.86	0.85
Ap297	184.00	11.00	2.76	0.58	1.00	0.00	0.00	0.64	0.64
Ap218	192.00	9.00	4.09	0.71	1.00	0.01	0.00	0.76	0.75
Ap001	184.00	21.00	7.56	0.85	0.39	0.01	0.52	0.87	0.86
A008	186.00	10.00	4.60	0.75	1.00	0.01	0.00	0.79	0.77
Ap226	196.00	7.00	2.29	0.50	1.00	0.01	0.00	0.57	0.56
Ap289	190.00	13.00	5.75	0.80	1.00	0.02	0.00	0.83	0.81
Ag005a	196.00	7.00	1.78	0.39	1.00	0.00	0.00	0.44	0.44
Mean	183.00	13.57	4.91	0.71	0.96	0.01	0.03	0.74	0.73

* N, number of total alleles used in each locus; N_A, number of observed alleles; N_E, number of effective allele; PIC, polymorphic information content; F_{IS}, inbreeding coefficient; F_{ST}, genetic differentiation coefficient; H_O, observed heterozygosis; H_E, expected heterozygosis; Mean H, mean heterozygosity.

The mean H_O value over all loci was found as 0.03. While the highest H_O value was 0.52 (Ap001), H_O was found to be 0 in other loci except Ap223, Ap238, Ap085, Ap001. In terms of H_E, the mean value was 0.74, highest and lowest values were 0.93 for Ap256 and 0.44 for Ag005a. These values (H_O and H_E) show the gene diversity in the Caucasian honey bee. Bodur et al. (2007) found that the mean H_O ranged from 0.52 (Eskişehir) to 0.67 (Cyprus) and the mean H_E ranged from 0.54 (Eskişehir) to 0.68 (Kastamonu) in 12 populations in Türkiye. İvgin Tunca (2009) found that the mean H_O ranged from 0.68 (Kars) to 0.38 (Artvin) and the mean H_E ranged from 0.45 (Muğla) to 0.74 (Artvin) in the honey bee populations in Türkiye. In a study examining genetic variability at eight microsatellite loci in *Apis mellifera ligustica* Spinola, 1806

(Hymenoptera: Apidae), the mean H_O was reported between 0.38 and 0.61, the mean H_E between 0.53 and 0.64 (Dall'Olio et al., 2007). In another study conducted on Lebanon honey bees, mean gene diversity was estimated to be 0.65 (Franck et al., 2000). Calculated values in terms of heterozygosity in this study are generally similar to the results reported in the literature. However, the observed heterozygosity was lower than the expected heterozygosity. This suggests that inbreeding has increased in protected colonies in the isolated region.

The indicators of genetic polymorphism and HWE values are given in Table 3 below for each two isolated regions. Statistics calculated for colonies in both isolated regions were found to be quite close to each other. While the F_{IS} and PIC values were higher in Artvin, the N_A, N_E, H_E and H_O values were higher in Ardahan.

Table 3. Main diversity parameters for each population

	Artvin	Ardahan
N	50.00	50.00
N_P	30.00	30.00
N_A	9.37	10.37
N_E	4.53	4.86
PIC	0.70	0.69
F_{IS}	0.98	0.93
H_E	0.74	0.74
H_O	0.02	0.04
HWE	0.00	0.00

* N, loci number; N_P, number of polymorphic loci N_A, number of observed alleles; N_E, number of effective allele; PIC, polymorphic information content; F_{IS}, coefficient of inbreeding; H_E, expected heterozygosis; H_O, observed heterozygosis; HWE, Hardy-Weinberg equilibrium ($p<0.01$).

Genetic differences for the two provincial populations were calculated using Arlequin with pairwise F_{ST} values developed by Weir & Cockerham (1984). According to this calculation, the two provinces are quite similar with only a slight difference (F_{ST}: 0.02). This study suggested that low levels of genetic differentiation were observed in isolated colonies, given the wide range of binary F_{ST} values previously reported (Garnery et al., 1998; Franck et al., 2000, 2001; Dall'Olio et al., 2007). Also, Nei's (1972) original genetic identity and genetic distance measurements were estimated and the genetic distance between populations was found to be 0.08. AMOVA analysis was performed to determine whether genetic variation was due to differentiation between populations or from individuals within the population. According to the results, the source of genetic variation was found to be 3.46% among populations, 96.54% among individuals within the population, and 0% for all individuals. As evident from these findings, Caucasian honey bee colonies under protection have the ability to represent the same race purely.

The differentiation between populations was also examined by Bayesian cluster analysis in Structure software (Figure 1). When K = 3, where the highest ΔK value is obtained, it is seen that phylogenetic relationships are best expressed and the Artvin (1) and Ardahan (2) populations are not clearly separated. Although same color bars representing individuals are found in both populations, it is seen that red segments are slightly more intense in the Ardahan (2) population. The fact that the colonies in the Artvin and Ardahan regions can be distinguished from each other, even if slightly, suggests that there are differences in the populations of the two provinces within the same protection area. The queen bees of the colonies in the two provinces obtained from different sources may cause this. However, the reasons for the differentiation in the Caucasian honey bee populations under protection in an isolated region should be investigated.

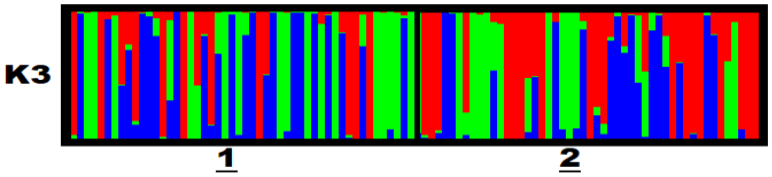

Figure 1. Genetic cluster analysis of Artvin and Ardahan populations. K represents the number of groups (1, Artvin; 2, Ardahan). The length of the colored bar represents the individual's membership coefficient in the cluster, according to cluster analysis.

As a result of the FCA analysis performed to reveal the phylogenetic relationships between the populations in three dimensions, the Artvin and Ardahan populations were separated from each other despite a pairwise F_{ST} value of 0.02 and interindividual variation was evident (Figure 2). The first axis explains 100% of the total variation.

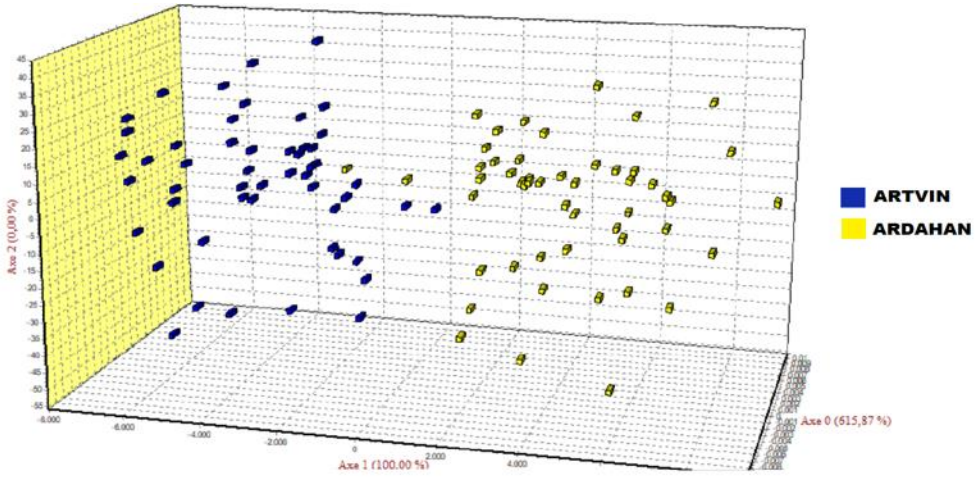

Figure 2. Factorial correspondence analysis of the Artvin and Ardahan populations on 30 polymorphic microsatellite loci. Each box represents a colony; the blue ones are Artvin and the yellow ones are Ardahan.

TPM is the most useful model, as mutations for microsatellite loci often do not yield consistent results with IAM or SMM (Dirienzo et al., 1994; Luikart et al., 1998; Piry et al., 1999; Fatima, 2006). TPM uses both IAM and SMM models together. Given that there are more than 20 loci, there appears to be a bottleneck in Caucasian honey bee populations according to Standardized differences test in the TPM model (Table 4) (Cornuet & Luikart, 1996). When the populations are evaluated separately as Artvin and Ardahan provinces, it is seen that the bottleneck is only present in Artvin (p<0.001), and there has not been a bottleneck in Ardahan recently (p>0.05).

The studied populations interestingly fit the GW index and the normal L-shaped distribution when the allele frequency distribution was analyzed with the qualitative graphical method defined by Luikart and Cornuet (1998) (Figure 3). However, the time and size of the bottleneck are effective in detecting a mode-shifted distribution. The fact that the bottleneck is not new or in the amount that can't be detected prevents a distorted distribution (Luikart et al., 1998; Wu et al., 2020). In addition, populations deviated from Hardy-Weinberg equilibrium (HWE) as a result of the disruption of genetic balance (p<0.01) (Table 3). Although deviation from HWE can be caused by many factors such as inbreeding, mutation and migration (Robertson & Hill, 1984), the deviation here supports the idea of the bottleneck brought about by inbreeding. The allele frequency distribution was analyzed graphically to determine whether it showed an L-shaped distribution. The microsatellite alleles were divided into 10 frequency classes, allowing us to determine whether the distribution shows the normal L-shaped form with an abundance of low-frequency alleles (0.01 to 0.1) (Luikart et al.,1998).

Table 4. Bottleneck analysis using two-phase mutation model

Statistical tests		TPM		
		Artvin population	Ardahan population	All populations
Sign test	EHE	17.75	17.74	17.63
	HD	18.00	16.00	25.00
	HE	12.00	14.00	5.00
	P	0.02658	0.11525	0.00000
Standardized differences test	T2	-3.130	-1.092	-7.683
	P	0.00087	0.13736	0.00000
Wilcoxon's signed rank test	HD (P)	0.02245	0.17461	0.00000
	HE (P)	0.97867	0.83063	1.00000
	HDE (P)	0.04491	0.34921	0.00001

* TPM, two-phased model; EHE, expected number of loci with heterozygosity excess; HD, one tail heterozygosity deficiency; HE, one tail heterozygosity excess; HDE, two tails for heterozygosity excess or deficiency; T2, standardized differences test. Positive values of the bottleneck statistic T2 are indicative of gene diversity excess caused by a recent reduction in effective population size, while negative values are consistent with a recent population expansion without immigration or immigration of some private alleles in the population.

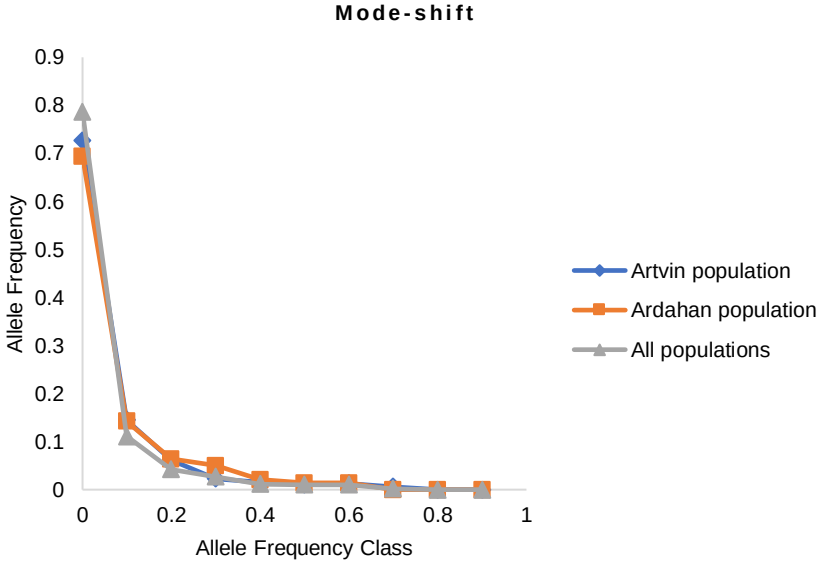

Figure 3. Allele frequency distribution of Artvin, Ardahan and all populations.

Such genetic bottlenecks are mostly associated with zoos, forest conditions, or relatively small and limited population size in isolated areas (Zhang et al., 2002; Luenser et al., 2005; Mukherjee et al., 2022). Although officially registered in Artvin and Ardahan provinces 138 362 hives (Gül & Nergiz, 2022); looking at the variation source in the Caucasian bee population, it was found that variation is insufficient and the variations come from among individuals, not among the populations. In the current situation, it would not be wrong to point out that there is no bee entry to the isolated area within the scope of the protection plan, that the queen bee is constantly supplied from the same source and that a bottleneck occurred after an inbreeding pressure caused by this. On the other hand, there are almost no studies on genetic factors, which are one of the most important factors affecting the quality of these queen bees, which are constantly supplied from the same source (Arslan et al., 2021). The founder effect, which is caused by the continuous use of queen bees from the same source, can also be shown as the cause of the bottleneck (Jamieson, 2011).

Bottlenecked populations have lost or are in danger of losing rare alleles, but there may still be some degree of heterozygosity (Luikart et al., 1998; Furlan et al., 2012; Ganapathi et al., 2012). Decreased genetic diversity and continued inbreeding can affect viability due to selection pressure as populations shrink in size (Al-Atiyat, 2008).

There are very few genetic characterization studies conducted on the Caucasian bee populations in Türkiye. Studies in the literature have reported that the Caucasian bee is in hybrid form in some regions and is in danger of losing its purity (Bodur, 2005; Kırpık et al., 2010). In this study, the genetic status of the Caucasian bee in the isolated regions of Artvin and Ardahan was determined. The results of the study show that the populations in the isolated region are generally Caucasian bees, but genetic diversity is beginning to be lost due to intensive inbreeding and there is evidence of genetic bottleneck in the Artvin population. However, it is understood from the Cluster and FCA analyses results that Artvin and Ardahan colonies differ in at least one locus. The reason for this may be that Artvin and Ardahan regions have different geographical and climatic environments and that the colonies here provide their queen bee needs from different sources. There is one queen bee production enterprise officially registered with the Ministry of Agriculture in the region, but there are also many unregistered practices that are not reported by beekeepers in the isolated region. Firstly, it is recommended to develop long-term strategies for programs that will reduce inbreeding and protect genetic diversity by increasing queen bee production enterprises in this isolated region. In addition, it is necessary to produce scientific outputs by ensuring that beekeeping activities in the region are wholly recorded and published regularly.

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