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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Microsatellite-Based Assessment of Genetic Variation and Demographic Bottlenecks in Kilis Goats Between Two Agro-Ecological Zones

İki Tarımsal-Ekolojik Bölgedeki Kilis Keçilerinde Mikrosatellit Belirteçlerle Genetik Çeşitlilik ve Demografik Darboğazların Değerlendirilmesi

Zühal GÜNDÜZ[1*], Nezih ATA[2], Onur YILMAZ[3], İbrahim CEMAL[4], Orhan KARACA[5], Mahmut KESKİN[6], Sabri GÜL[7], Hatice YILMAZ TİLKİ[8]

Abstract

The Kilis goats, one of Türkiye's prominent indigenous goat breeds, are extensively raised in the southern regions of the country due to its exceptional milk production potential. This study aimed to investigate the population dynamics of the Kilis goats, which are renowned for their high milk yield and remarkable adaptability, at the molecular genetic level. A total of 190 individuals from Kilis goat populations raised in two different provinces were characterized using 16 microsatellites. A total of 274 alleles were detected across all loci studied. The results for the mean number of alleles, expected heterozygosity, and polymorphic information content were 17.13, 0.85, and 0.83, respectively. It was revealed that 99.0% of the total genetic variation can be attributed to genetic differences among individuals, while only 1.0% can be explained by differences among populations. The F_{ST} value obtained (0.022) indicates a low level of genetic differentiation between the studied populations. These findings consistently indicate a high degree of genetic similarity and effective gene flow between the two provincial populations. STRUCTURE analysis revealed the presence of three distinct genetic clusters among the studied populations, indicating intra-breed sub structuring rather than clear inter-population divergence. Bottleneck analysis the analysis produced an L-shaped curve in the allele frequency distribution, indicating no significant recent genetic bottleneck in the studied populations. This study provides important insights into the genetic diversity and population structure of Kilis goat populations raised in the Gaziantep and Kilis provinces. The results demonstrate that the Kilis goat population exhibits high genetic diversity. Overall, the results highlight substantial within-breed genetic diversity, low inter-population differentiation, and the absence of a recent demographic bottleneck, providing a scientific foundation for sustainable genetic management.

Keywords: Kilis goats, Fertile crescent, Genetic diversity, Small ruminant

[1]***Sorumlu Yazar/Corresponding Author:** Zühal Gündüz, Aydin Adnan Menderes University Faculty of Agriculture Department of Agricultural Biotechnology, Aydın, Türkiye. E-mail: zuhal.gunduz@adu.edu.tr OrcID: 0000-0002-1427-1501

[2]Nezih Ata, Aydin Adnan Menderes University Faculty of Agriculture Department of Animal Science, Aydin, Türkiye. E-mail: nezihata@adu.edu.tr OrcID: 0000-0002-0387-5131

[3]Onur Yılmaz, Aydin Adnan Menderes University Faculty of Agriculture Department of Animal Science, Aydin, Türkiye. E-mail: oyilmaz@adu.edu.tr OrcID: 0000-0002-5658-8558

[4]İbrahim Cemal, Aydin Adnan Menderes University Faculty of Agriculture Department of Animal Science, Aydin, Türkiye. E-mail: cemal_i@yahoo.com OrcID: 0000-0002-4069-4815

[5]Orhan Karaca, Aydin Adnan Menderes University Faculty of Agriculture Department of Animal Science, Aydin, Türkiye. E-mail: karaca_o@yahoo.com OrcID: 0000-0002-7323-9173

[6]Mahmut Keskin, Hatay Mustafa Kemal University Faculty of Agriculture Department of Animal Science, Hatay, Türkiye. E-mail: mkeskin@mku.edu.tr OrcID: 0000-0002-8147-2477

[7]Sabri Gül, Hatay Mustafa Kemal University Faculty of Agriculture Department of Animal Science, Hatay, Türkiye. E-mail: sabrigul@gmail.com OrcID: 0000-0001-6787-8190

[8]Hatice Yılmaz Tilki, Hatay Mustafa Kemal University Graduate School of Natural and Applied Sciences, Hatay, Türkiye. E-mail: hatice.tilkiyilmaz@tarimorman.gov.tr OrcID: 0000-0002-9308-2169

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Öz

Türkiye'nin önemli yerli keçi ırklarından biri olan Kilis keçisi, yüksek süt verim potansiyeli nedeniyle özellikle ülkenin güney bölgelerinde yaygın olarak yetiştiriciliği yapılmaktadır. Bu çalışmada, yüksek süt verimi ve dikkat çekici adaptasyon yeteneği ile bilinen Kilis keçilerinin popülasyon dinamiklerinin moleküler genetik düzeyde araştırılması amaçlanmıştır. Çalışmada, iki farklı ilde yetiştirilen Kilis keçisi popülasyonlarına ait toplam 190 birey, 16 mikrosatellit belirteç kullanılarak incelenmiştir. İncelenen tüm lokuslarda toplam 274 allel tespit edilmiştir. Ortalama allel sayısı, beklenen heterozigotluk ve polimorfik bilgi içeriği bakımından değerler sırasıyla 17.13, 0.85 ve 0.83 olarak tespit edilmiştir. Toplam genetik varyasyonun %99.0'ının bireyler arası genetik farklılıklardan kaynaklandığı, yalnızca %1.0'ının ise popülasyonlar arasındaki farklılıklarla açıklanabildiği ortaya konulmuştur. Elde edilen F_{ST} değeri (0.022), incelenen popülasyonlar arasında düşük düzeyde genetik farklılaşma olduğunu göstermektedir. Bu bulgular, iki ildeki popülasyonlar arasında yüksek düzeyde genetik benzerlik ve etkin gen akışı bulunduğunu tutarlı biçimde ortaya koymaktadır. STRUCTURE analizi, incelenen popülasyonlarda üç farklı genetik kümenin varlığını ortaya koymuş olup, bu durum belirgin popülasyonlar arası ayrışmadan ziyade ırk içi alt yapılanmaya işaret etmektedir. Bottleneck analizi sonucunda, allel frekans dağılımında L-şekilli bir eğri elde edilmiş ve bu durum, incelenen popülasyonlarda yakın geçmişte önemli bir genetik darboğaz yaşanmadığını göstermiştir. Bu çalışma, Gaziantep ve Kilis illerinde yetiştirilen Kilis keçisi popülasyonlarının genetik çeşitliliği ve popülasyon yapısı hakkında önemli bilgiler sunmaktadır. Elde edilen sonuçlar, Kilis keçisi popülasyonunun yüksek genetik çeşitlilik sergilediğini ortaya koymaktadır. Genel olarak bulgular, yüksek düzeyde ırk içi genetik çeşitlilik, düşük popülasyonlar arası farklılaşma ve yakın geçmişte demografik bir darboğazın bulunmadığını göstermekte olup, Kilis keçisinin sürdürülebilir ıslah stratejileri için bilimsel bir temel sağlamaktadır.

Anahtar Kelimeler: Kilis keçisi, Bereketli hilal, Genetik çeşitlilik, Küçükbaş hayvan

1. Introduction

Goat husbandry plays a crucial role in Türkiye's livestock production industry, supported by the country's rich agricultural heritage and diverse ecological conditions (Gül et al., 2020; Gündüz and Biçer, 2023). As of 2024, the goat population stands at 10.3 million, making this sector a significant contributor to the national agricultural economy. This highlights the strategic importance of goat production in sustaining rural livelihoods and promoting environmentally adapted farming systems (Anonymous, 2024).

Kilis goats, primarily raised in the southern regions of Türkiye using extensive breeding systems, is predominantly found in the provinces of Kilis, Gaziantep, Hatay, and Sanliurfa (Keskin et al., 2017; Gül et al., 2020; Gündüz and Biçer, 2023). The Kilis goat is recognized for its relatively high milk yield compared to other indigenous breeds and its ability to efficiently utilize low-quality forage under extensive production systems. This breed exhibits favorable lactation performance, good reproductive efficiency, and strong adaptability to the hot, semi-arid conditions of Southern Anatolia. Its tolerance to nutritional limitations, climatic stress, and traditional management practices makes the Kilis goat a valuable genetic resource for sustainable small ruminant production in marginal environments (Gül et al., 2016; Daşkıran et al., 2022; Gündüz and Biçer, 2023; Erduran, 2023; Erduran and Boztepe, 2025). It is reported by breeders that the Kilis goats, primarily raised in regions of Türkiye close to the Syrian border, originated from unsystematic crossbreeding between the Syrian Damascus goat and the Turkish Hair goat (Keskin et al., 2017). While initially based on breeder observations, recent molecular studies have provided genetic evidence supporting this hypothesis. Using microsatellites, Gül et al. (2020) demonstrated that Kilis goats exhibit a strong genetic affinity with the Damascus goats and show patterns consistent with admixture between Damascus and Turkish Hair goats, supporting the hypothesis of a composite origin. Additionally, mitochondrial DNA analyses conducted by Karaköse and Gündüz (2025) further clarified the maternal lineage of the Kilis goats, revealing haplotypic relationships closely associated with Damascus goat populations. Taken together, these findings provide a clearer genetic framework for understanding the composite origin and evolutionary history of the Kilis goats (Gül et al., 2020; Karaköse and Gündüz, 2025).

After the breed was incorporated into “The National Genetic Improvement Project for Small Ruminants under Breeders' Conditions”, initiated by the Turkish Ministry of Agriculture and Forestry in 2005, a modern breeding program was implemented for Kilis goats (Daşkıran et al., 2022; Daşkıran, 2024). Within the scope of the breeding program, it has become possible to maintain yield and pedigree records. While the documentation of these records and the implementation of modern breeding systems in the field are primary objectives of breeding programs, identifying both intra- and inter-breed genetic structures is also a crucial factor in achieving these goals (Karaca et al., 2014).

In recent years, advancements in bioinformatics and molecular genetics have enabled the identification of genetic similarities and differences within and between breeds using molecular markers. Among these markers, microsatellites (short tandem repeats, STRs) are short nucleotide motifs (1–6 base pairs) and highly informative, co-dominant, PCR-based markers consisting of short, repeated DNA motifs widely distributed across eukaryotic genomes (Katti et al., 2001). Due to their high mutation rate, locus specificity, and widespread distribution throughout the genome, microsatellites provide detailed insights into genomic structure, allelic richness, heterozygosity, and population-level genetic variation. The primary applications of microsatellites include assessing genetic diversity, analyzing population structure, parentage testing, breed characterization, conservation genetics, detecting inbreeding levels, and identifying historical demographic events such as population bottlenecks and expansions. Their multi-allelic nature confers high discriminatory power compared to bi-allelic markers, making them particularly suitable for fine-scale population genetic studies (Toth et al., 2000; Katti et al., 2001; Li et al., 2004; Srivastava et al., 2019). These markers provide exceptional genetic variability, making them invaluable for population genetic analyses (Togan et al., 2005; Gündüz et al., 2016). Compared to other genetic markers such as single nucleotide polymorphisms (SNPs) and mitochondrial DNA, microsatellites offer several advantages, including higher polymorphism per locus, strong informativeness for recent evolutionary events, co-dominant inheritance, cost-effectiveness, and suitability for studies with moderate sample sizes. While SNP markers provide extensive genome-wide coverage, microsatellites remain superior for detecting recent genetic bottlenecks, assessing within-breed diversity, and conducting detailed population structure analyses in livestock species (Vignal et al., 2002; Borsting et al., 2016). Due to these properties, microsatellites are widely utilized for

identifying genetic diversity in livestock and conducting bottleneck tests (Gül et al., 2020; Demiray et al., 2024; Cemal et al., 2024). Overall, their high resolution, reproducibility, and analytical efficiency make microsatellites a powerful and practical tool for conservation, genetic resource management, and optimization of breeding strategies in farm animals. In recent years, high-density SNP panels and next-generation sequencing approaches have been increasingly applied in population genetic studies because of their genome-wide coverage and ability to detect fine-scale population structure (Demir et al., 2023; Bayraktar et al., 2024; Bilginer et al., 2025). However, for studies primarily focused on intra-breed diversity, recent demographic signals, and heterozygosity patterns, microsatellites remain a robust and cost-effective alternative, especially in managed livestock populations.

The primary aim of this study was to characterize the intra-breed genetic structure of Kilis goats using microsatellite markers. To achieve this, the study was designed around three specific objectives: (i) to quantify genetic diversity within the population; (ii) to evaluate population structure and the extent of genetic differentiation; and (iii) to detect potential demographic signals, particularly genetic bottlenecks, that may have influenced the current population structure. Despite the recognized economic and adaptive importance of the Kilis goat in Türkiye, comprehensive assessments of its intra-breed genetic structure remain limited. Therefore, elucidating the genetic diversity, population structure, and demographic history of this breed is essential for developing sustainable breeding strategies, conserving genetic resources, and implementing informed management practices.

2. Materials and Methods

Ethical approval for all procedures involving animals was granted by the Animal Experiments Local Ethics Committee of Aydin Adnan Menderes University, with authorization documented under protocol number 64583101/2021/181.

2.1. Sample collection and DNA extraction

The study's animal material comprised 190 Kilis goats from farms participating in the “Kilis Goat Breeding” sub-project within the scope of “The National Genetic Improvement Project for Small Ruminants under Breeders' Conditions” implemented by TAGEM in Gaziantep and Kilis provinces of Türkiye. A total of 10 independent flocks were included in the study, with five flocks sampled from each province. Approximately 19 animals were randomly selected from each flock, resulting in a total sample size of 190 individuals. Flocks were chosen to represent the active breeding units within the project framework, and within-flock sampling was conducted randomly to reflect the existing genetic composition under field conditions. This sampling approach aimed to ensure geographic representativeness while minimizing potential bias from the overrepresentation of specific management units. The criteria specified by the FAO (Anonymous, 2011) were considered in determining the sampling strategy for the study. Blood samples, approximately 9 mL each, were obtained from the animals' jugular vein via vacuum tubes preloaded with the anticoagulant ethylenediaminetetraacetic acid (EDTA; BD Vacutainer Systems, Plymouth, UK). Nuclear DNA was meticulously extracted from leukocytes using the salting out method described by Miller et al., (1988) and Montgomery and Sise, (1990) and kept at −20°C for subsequent analyses. The quality and quantity of the DNA obtained were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

2.2. PCR and fragment analysis

For this analysis, 16 microsatellite primers were selected following the guidelines outlined in the “Molecular Genetic Characterization of Animal Genetic Resources” by the Food and Agriculture Organization (FAO, 2011). The selection criteria included high polymorphism information content (PIC), clear and reproducible amplification profiles, suitability for multiplex PCR design, and broad distribution across different chromosomes to avoid linkage bias and ensure comprehensive genome-wide representation. The microsatellites used in this study were originally developed for closely related species and applied to goats through cross-species amplification. All selected loci had been previously validated in goat genetic studies, with their amplification success, polymorphism, and reproducibility confirmed prior to analysis (Tefiel et al., 2018; Keskin et al., 2019; Gül et al., 2020). The primers utilized in this study were labeled with fluorescent dyes compatible with the Beckman Coulter GeXP device (Beckman Coulter, Inc., USA). The corresponding GenBank accession numbers and locus-related information are provided in *Table 1*.

Table 1. Microsatellite loci and primer information

Multiplex	Dye	Microsatellites (Accession numbers)		Primer sequences
M1	D2	OarAE54 (L11048)	**F**	TACTAAAGAAACATGAAGCTCCCA
			R	GGAAACATTTATTCTTATTCCTCAGTG
	D2	BM1818 (G18391)	**F**	AGCTGGGAATATAACCAAAGG
			R	AGTGCTTTCAAGGTCCATGC
	D3	OarFCB20 (L20004)	**F**	AATGTGTTTAAGATTCCATACAGTG
			R	GGAAAACCCCCATATATACCTATAC
	D3	INRA0005 (X63793)	**F**	CAATCTGCATGAAGTATAAATAT
			R	CTTCAGGCATACCCTACACC
	D3	INRA0023 (X67830)	**F**	GAGTAGAGCTACAAGATAAACTTC
			R	TAACTACAGGGTGTTAGATGAACTC
	D3	ILSTS011 (L23485)	**F**	GCTTGCTACATGGAAAGTGC
			R	CTAAAATGCAGAGCCCTACC
	D4	SRCRSP9 (L22201)	**F**	AGAGGATCTGGAAATGGAATC
			R	GCACTCTTTTCAGCCCTAATG
	D4	TCRVB6 (L18953)	**F**	GAGTCCTCAGCAAGCAGGTC
			R	CCAGGAATTGGATCACACCT
M2	D3	McM0527 (L34277)	**F**	GTCCATTGCCTCAAATCAATTC
			R	AAACCACTTGACTACTCCCCAA
	D3	CSRD0247 (---)	**F**	GGACTTGCCAGAACTCTGCAAT
			R	CACTGTGGTTTGTATTAGTCAGG
	D4	SRCRSP0023 (---)	**F**	TGAACGGGTAAAGATGTG
			R	TGTTTTTAATGGCTGAGTAG
	D4	ILSTS0087 (L37279)	**F**	AGCAGACATGATGACTCAGC
			R	CTGCCTCTTTTCTTGAGAG
	D4	SRCRSP0005 (L22197)	**F**	GGACTCTACCAACTGAGCTACAAG
			R	TGAAATGAAGCTAAAGCAATGC
	D4	DRBP1 (M55069)	**F**	ATGGTGCAGCAGCAAGGTGAGCA
			R	GGGACTCAGTCTCTCTATCTCTTTG
	D4	INRABERN172 (---)	**F**	CCACTTCCCTGTATCCTCCT
			R	GGTGCTCCCATTGTGTAGAC
	D4	HSC (M90759)	**F**	CTGCCAATGCAGAGACACAAGA
			R	GTCTGTCTCCTGTCTTGTCATC

The PCR reaction mixture totaled 20 µL and included 1X PCR buffer, 2.0 mM MgCl2, 0.20 mM dNTPs (dATP, dTTP, dGTP, dCTP), 0.10 µM of each forward and reverse primer, 1 U of Taq DNA polymerase (Thermo Scientific, USA) and 50 ng template DNA. DNA amplification was conducted using the touchdown PCR technique as outlined by Hecker and Roux (1996). The thermal cycling conditions was given in *Table 2*. The PCR reactions were carried out on a C1000 Touch™ thermal cycler (Bio-Rad, USA). The fluorescently labeled PCR products were resolved through capillary electrophoresis using the Beckman Coulter GeXP Genetic Analyzer (Beckman Coulter, Inc., USA), ensuring precise separation and analysis.

Table 2. Thermal cycler protocols utilizing the Touchdown PCR method

Multiplex groups	First denaturation	Denaturation	Annealing	Extension	Final extension	Total cycle
M1	95 ºC (5 min)	95 ºC (40 sec)	60-50 ºC (40 sec)	72 ºC (60 sec)	72 ºC (5 min)	36
M2	95 oC (5 min)	95 oC (40 sec)	60-50 ºC (40 sec)	72 ºC (60 sec)	72 ºC (5 min)	36

2.3. Statistical analysis

The genetic diversity within the population was assessed through various metrics, including the total number of alleles (Na), mean number of allele (MNa), effective allele number (Ne), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), and Wright's fixation indices (F_{IT}, F_{IS}, F_{ST}) (Weir and Cockerham, 1984; Wright, 1990). Additionally, deviations of populations from the Hardy-Weinberg equilibrium were evaluated. Data analyses were conducted using GenAlEx (Peakall and Smouse, 2012) and CERVUS 3.0.3 (Marshall,

2006; Kalinowski et al., 2007). The coefficient of gene differentiation (G_{ST}) was estimated with FSTAT 2.9.3 (Goudet, 2001). To further investigate population structure, a Bayesian clustering approach was implemented using STRUCTURE (Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009). The analysis employed the admixture model with independent allele frequencies, applying a burn-in period of 20,000 iterations followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations. For each potential number of clusters (K = 2–4), 20 independent replicates were performed to ensure consistency. The range of K values was conservatively limited to 2-4 to identify meaningful genetic sub-structuring while avoiding the risk of over-partitioning within the dataset. Since the study focuses on the intra-breed genetic structure of a single population sampled from a limited number of flocks, high levels of ancestral differentiation were not anticipated. Testing higher K values in such a genetically homogeneous context can lead to the artificial subdivision of individual genotypes into spurious clusters that reflect statistical noise or familial relationships rather than true biological or evolutionary divergence (Pritchard et al., 2000). The optimal number of clusters was subsequently determined using the Evanno method (Evanno et al., 2005), which evaluates the second-order rate of change in the log probability of the data between successive K values. The STRUCTURE results were summarized and visualized using alignment plots generated by CLUMPAK (Kopelman et al., 2015). The most likely number of clusters was identified with STRUCTURE HARVESTER (Earl and vonHoldt, 2012) by applying the ΔK method of Evanno et al. (2005), calculated as $\Delta K = m|L''(K)|/s[L(K)]$. To investigate potential genetic bottlenecks within the populations, analyses were conducted using Bottleneck software version 1.2.0.2 (Piry et al., 1999). The evaluation was performed under three mutational models: the Infinite Allele Model (IAM), the Stepwise Mutation Model (SMM), and the Two-Phase Mutation Model (TPM). Statistical significance was assessed using the Sign test, Standardized differences test, and Wilcoxon signed-rank test, based on 1,000 simulation replicates to ensure robustness and reliability (Luikart et al., 1998; Piry et al., 1999).

3. Results

A total of 274 alleles were identified across 16 microsatellite loci, with the corresponding molecular genetic polymorphism parameters presented in *Table 3*. Among these loci, BM1818 exhibited the greatest allelic richness, harboring 29 alleles, along with the highest effective number of alleles (18.64). This locus also showed the maximum polymorphic information content (PIC) value of 0.94, highlighting its high discriminatory power. Expected heterozygosity (He) values ranged from 0.73 at ILSTS87 to 0.94 at BM1818, while observed heterozygosity (Ho) varied from 0.54 at ILSTS87 to 0.90 at INRABERN172. The average Wright's fixation indices were estimated as 0.165 for F_{IS}, 0.183 for F_{IT}, and 0.022 for F_{ST}. The F_{IS} value (0.165) indicates a deficit of heterozygotes within subpopulations, suggesting non-random mating patterns and a measurable level of inbreeding under the current breeding structure. Specifically, the positive F_{IS} value indicates increased homozygosity within flocks, consistent with the use of a limited number of breeding males and flock-based mating systems that increase relatedness among individuals. The corresponding F_{IT} value (0.183) reflects an overall reduction in heterozygosity at the total population level. In contrast, the low F_{ST} value (0.022) indicates low genetic differentiation, suggesting substantial gene flow and a largely shared gene pool. Additionally, the mean coefficient of gene differentiation (G_{ST}) was calculated as 0.011, further confirming minimal divergence among populations. The low G_{ST} value (0.011) indicates a high degree of genetic homogeneity among the studied flocks, with only a negligible fraction of the total genetic diversity (1.1%) attributable to differences between subpopulations. This suggests that most of the genetic variation is partitioned within flocks, likely due to historical gene flow or the shared genetic background of the sampled individuals. Taken together, these parameters indicate that, although overall genetic differentiation between provinces is low, detectable intra-breed genetic structuring exists. This structuring is likely driven by mating patterns and management practices rather than by true population separation.

Hardy–Weinberg equilibrium (HWE) was assessed for the 16 microsatellite loci using the χ^2 test (*Table 3*). The results revealed significant deviations from HWE at all loci. These deviations can arise from two main sources: biological processes (e.g., non-random mating, population sub structuring, or inbreeding) and methodological factors (e.g., genotyping errors or null alleles). However, the null allele frequencies in the present study remained below the accepted threshold, indicating that technical artifacts are unlikely to explain the observed deviations. These deviations are likely associated with population sub structuring and non-random mating patterns within the sampled populations, as indicated by the positive F_{IS} values observed across most loci. Moreover, the consistently higher expected heterozygosity (He) compared to observed heterozygosity (Ho) suggests a deficit of heterozygotes, which may result from factors such as population stratification, assortative mating, or breeding practices within

local flocks. In addition, null allele frequencies were below the commonly accepted 20% threshold (Dakin and Avise, 2004) for all loci, indicating that the observed deviations from HWE are unlikely to result from genotyping artifacts. Therefore, methodological explanations can largely be excluded, and the deviations from HWE are more plausibly attributed to biological factors, particularly mating structure and flocks-based breeding practices.

Table 3. Polymorphism statistics for the microsatellite loci

Locus	Na	Ne	Ho	He	PIC	G_{ST}	F_{IS}^*	F_{IT}^*	F_{ST}^*	F(Null)	HWE	
											Prob	Sign.
OarAE54	16.00	5.19	0.71	0.81	0.79	0.023	0.169	0.186	0.020	0.0676	0.000	***
BM1818	29.00	18.64	0.70	0.95	0.94	0.010	0.158	0.176	0.022	0.1489	0.000	***
OarFCB20	17.00	5.05	0.63	0.80	0.78	0.034	0.163	0.179	0.019	0.1343	0.000	***
INRA5	14.00	8.41	0.80	0.88	0.87	0.013	0.170	0.188	0.022	0.0488	0.000	***
INRA23	25.00	13.36	0.88	0.93	0.92	0.004	0.173	0.192	0.023	0.0261	0.000	***
ILSTS11	17.00	5.11	0.67	0.80	0.78	-0.001	0.164	0.183	0.023	0.0943	0.000	***
SRCRSP9	19.00	7.91	0.66	0.87	0.86	0.004	0.159	0.179	0.023	0.1402	0.000	***
TCRVB6	17.00	10.43	0.78	0.90	0.90	-0.002	0.166	0.186	0.024	0.0755	0.000	***
McM527	10.00	4.59	0.56	0.78	0.76	0.005	0.157	0.176	0.023	0.1775	0.000	***
CSRD247	14.00	6.97	0.68	0.86	0.84	0.006	0.162	0.181	0.023	0.1186	0.000	***
SRCRSP23	15.00	6.43	0.58	0.84	0.83	0.028	0.156	0.173	0.020	0.1919	0.000	***
ILSTS87	15.00	3.65	0.54	0.73	0.71	0.011	0.160	0.178	0.022	0.1553	0.000	***
SRCRSP5	13.00	6.26	0.69	0.84	0.82	0.001	0.163	0.183	0.023	0.0972	0.000	***
DRBP1	23.00	8.84	0.70	0.89	0.88	0.040	0.163	0.178	0.018	0.1203	0.000	***
INRABERN172	12.00	3.97	0.90	0.75	0.72	0.005	0.186	0.204	0.023	-0.1139	0.000	***
HSC	18.00	10.39	0.75	0.90	0.90	0.001	0.164	0.183	0.023	0.0936	0.000	***
Mean	17.13	7.82	0.70	0.85	0.83	0.011	0.165	0.183	0.022			

Na: Number of alleles, **Ne:** Effective number of alleles, **Ho:** Observed heterozygosity, **He:** Expected heterozygosity, **PIC:** Polymorphic information content, **G_{ST}:** Coefficient of gene differentiation, ***:** Wright's statistics according to Weir and Cockerham (1984), **F(Null):** Null allele frequency, **HWE:** Significance level of Hardy-Weinberg Equilibrium, ***:** P<0.05, *******: P<0.001

Detailed genetic diversity parameters for Kilis goat populations from Gaziantep and Kilis regions are summarized in *Table 4*.

Table 4. Polymorphism statistics of Kilis goat populations raised in different locations

Locations	MNA	Mean Heterozygosity		F_{IS}	HWE	NPA		Total
		Ho (SE)	He (SE)			Freq. ≥5%	Freq. <%5	
Gaziantep	14.94	0.66	0.83	0.205	14	2	44	46
Kilis	14.25	0.73	0.83	0.126	15	5	30	35

MNA: Mean number of alleles, **Ho:** mean observed heterozygosity, **He:** mean expected heterozygosity, **F_{IS}:** within-breed heterozygote deficiency, **HWE:** number of loci not in the Hardy- Weinberg equilibrium (P < 0.05), **NPA:** number of private alleles

The Kilis goat populations from Gaziantep and Kilis exhibited mean numbers of alleles (MNA) of 14.94 and 14.25, respectively. Observed heterozygosity (Ho) was lower than expected heterozygosity (He) in both populations (0.66 vs. 0.83 for Gaziantep and 0.73 vs. 0.83 for Kilis), accompanied by positive F_{IS} values (0.205 and 0.126, respectively). This indicates a heterozygote deficit, which may reflect population substructure or non-random mating within the sampled populations. A total of 81 private alleles were detected across the two populations, with 46 observed in Gaziantep and 35 in Kilis. However, only a small proportion of these alleles occurred at frequencies higher than 5% (two alleles in Gaziantep and five in Kilis), while the majority were present at low frequencies. This pattern suggests that although private alleles contribute to the overall genetic diversity of the populations, their low frequencies indicate limited differentiation and a substantial level of shared genetic variation between the two populations.

The findings from the STRUCTURE analysis, which considered a range of clustering values (K=2 to K=4), are depicted in *Figure 1*.

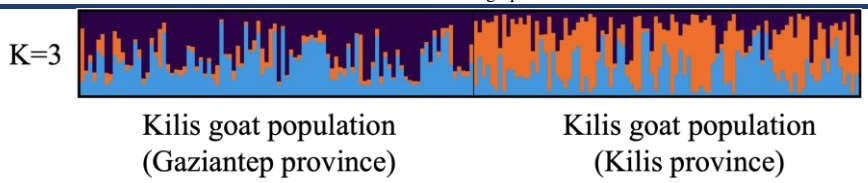


Figure 1. CLUMPAK Visualization of Population Structure in Kilis Goats Based on STRUCTURE Assignments for K = 2–4

The results, including the posterior probability estimates ([Ln Pr(X|K)]) for determining the number of clusters (K) and the associated ΔK values, are summarized in *Table 5*.

Table 5. Estimated posterior probabilities [Ln Pr(X|K)] and ΔK values for different STRUCTURE clustering scenarios in Kilis goat populations

K	Reps	Mean LnP(K)	ΔK
2	20	-11.933	----
3	20	-12.049	1.81
4	20	-12.036	----

STRUCTURE analysis revealed overlapping genetic patterns among the examined populations. Utilizing the approach described by Evanno et al. (2005), the ΔK values clearly indicated that three is the best number of genetic clusters to represent the data. To evaluate the potential occurrence of genetic bottlenecks, the dataset was analyzed using three distinct mutation models, in line with the guidelines of Luikart et al. (1998) and Piry et al. (1999). These models included the Infinite Allele Model (IAM), the Stepwise Mutation Model (SMM), and the Two-Phase Mutation Model (TPM). The detailed results of the bottleneck analysis are presented in *Table 6*.

Table 6. Genetic bottleneck assessment in Kilis goat populations under different mutation models

Location	Mutation Models	Sign test				Standardized differences test		Wilcoxon rank test (one tail for H excess)
		Hee	Hed	He	P	T2	P	P
Gaziantep	IAM	9.69	3	13	0.07122	2.688	0.00359	0.00105
	TPM	9.31	5	11	0.27753	-0.860	0.19485	0.46994
	SMM	9.44	14	2	0.00017	-7.988	0	0.99981
Kilis	IAM	9.72	2	14	0.02102	3.340	0.00042	0.00005
	TPM	9.48	4	12	0.15131	0.347	0.3644	0.16125
	SMM	9.40	14	2	0.00019	-5.965	0	0.99986

IAM: Infinite Allele Model, **SMM:** Stepwise Mutation Model, **TPM:** Two Phase Mutation Model **Hee:** Expected number of loci with heterozygosity excess, **Hed:** heterozygosity deficiency, **He:** heterozygosity excess

Potential genetic bottlenecks in Kilis goat populations have been visually demonstrated using a mode-shift graph (*Figure 2*).

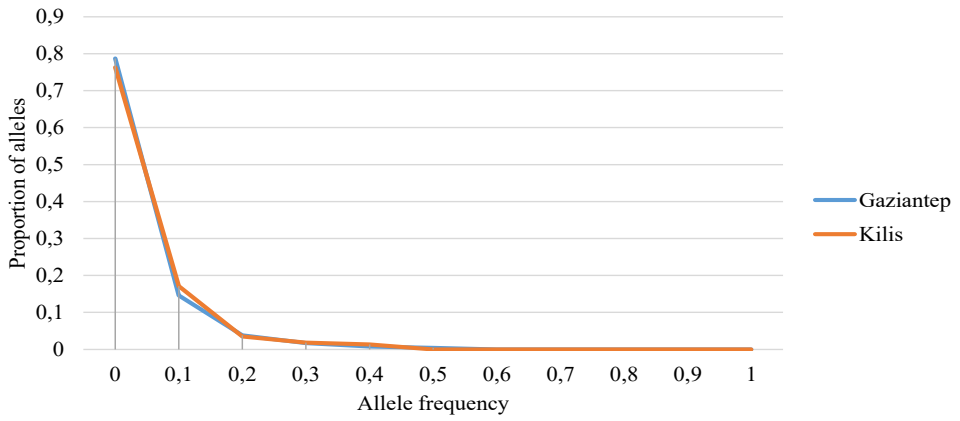


Figure 2. Mode-shift analysis of allele frequency distribution for detecting genetic bottlenecks in Kilis goat populations

Bottleneck analysis of the Kilis goat populations revealed no evidence of a recent demographic contraction. The allele frequency distribution displayed the characteristic L-shaped pattern (*Figure 2*), with most alleles occurring at low frequencies, indicative of populations at mutation–drift equilibrium and lacking recent reductions in effective population size (Luikart et al., 1998). The Two-Phase Mutation Model (TPM) was employed as the primary framework for bottleneck detection, as it is widely recognized as the most biologically realistic model for microsatellite loci, integrating both single-step and multi-step mutation processes (Luikart et al., 1998; Piry et al., 1999). Statistical significance was evaluated using the Wilcoxon test, which is suitable for studies with a moderate number of loci. To ensure methodological robustness and comparability with previous research, the Infinite Allele Model (IAM) and Stepwise Mutation Model (SMM) were also analyzed. Reporting IAM and SMM results alongside TPM provides a comparative framework for evaluating the sensitivity of bottleneck detection to different mutation assumptions. In the Kilis goat populations, consistent outcomes across all three models reinforce the conclusion that no recent genetic bottleneck has occurred, reflecting the maintenance of mutation–drift equilibrium. This approach aligns with established practices in microsatellite-based bottleneck studies (Di Rienzo et al., 1994; Luikart et al., 1998; Piry et al., 1999).

4. Discussion

Overall, the findings of this study indicate that Kilis goat populations maintain high allelic diversity and low inter-population differentiation, while exhibiting low intra-population inbreeding and heterozygote deficiency. In line with the main objective of the study, these results provide a comprehensive picture of the current intra-breed genetic structure of the Kilis goats.

The Kilis goat populations exhibited a higher number of alleles, and a greater average polymorphic information content (PIC) compared to those reported in similar studies (Kawecka et al., 2022; Lai et al., 2023; Pakpahan et al., 2023; Whannou et al., 2023; Zhao et al., 2023; Yordanov et al., 2024; Cemal et al., 2024). The results of this study highlight the remarkable informativeness and discriminatory power of the 16 microsatellite loci analyzed. The high levels of average heterozygosity and mean allelic richness per locus provide compelling evidence of the extensive genetic diversity maintained within the examined goat populations. This pronounced polymorphism not only validates the suitability of the selected microsatellites for population genetic studies but also demonstrates their effectiveness in accurately capturing the underlying genetic structure. Although the observed (Ho) and expected (He) heterozygosity values in the current population were lower than those reported in some previous studies (Kawecka et al., 2022; Zhao et al., 2023; Demiray et al., 2024), other investigations have documented higher estimates (Muner et al., 2021; Erniee Muneerah et al., 2021; Mukhina et al., 2022; Pakpahan et al., 2023; Whannou et al., 2023; Lai et al., 2023), reflecting both the population-specific nature of genetic diversity and the influence of historical management practices. Taken together, these comparisons indicate that the Kilis goat population retains considerable within-breed variation while also reflecting the effects of its own breeding history and management conditions. Collectively, these results provide a robust foundation for understanding the genetic composition of Kilis goat populations and reinforce the critical role of highly polymorphic microsatellites in guiding conservation and breeding strategies.

The region where the Kilis goats are raised is part of the Fertile Crescent, one of the world's most significant centers of domestication and renowned for its rich genetic reservoir. It is believed that other local goat populations, such as the indigenous Hair goat and the Damascus goat of Syrian origin, have contributed to the development of the Kilis goats (Gül et al., 2020; Gündüz, 2025; Karaköse and Gündüz, 2025). Furthermore, uncontrolled and non-systematic crossbreeding practices conducted by local farmers among these breeds have further shaped the existing gene pool in the region. Therefore, the genetic diversity parameters identified in the present study for the Kilis goat can be considered a reflection of the breed's current genetic background within this geographical context.

Wright's F-statistics (F_{IS}, F_{IT}, F_{ST}) (Weir and Cockerham, 1984; Wright, 1990) serve as essential metrics for assessing genetic variability, population dynamics, and evolutionary processes. In this study, the F-statistics obtained from microsatellites provide valuable insights into the genetic structure of populations. The average F_{IS} value (0.165) indicates a higher-than-expected level of homozygosity within populations, reflecting a moderate level of inbreeding among individuals. The F_{IS} values reported here are higher than those documented in several previous studies (Mukhina et al., 2022; Kawecka et al., 2022; Pakpahan et al., 2023; Whannou et al., 2023; Zhao et al., 2023; Lai et al., 2023; Yordanov et al., 2024; Demiray et al., 2024), yet significantly lower than those found

in others (Erniee Muneerah et al., 2021; Cemal et al., 2024). Conversely, the low F_{ST} value (0.022) suggests minimal genetic differentiation among Kilis goat populations across different provinces, indicating a largely homogeneous genetic structure. Therefore, the primary threat to genetic diversity conservation in these populations arises not from interpopulation isolation but from inbreeding pressure within populations. The F_{ST} value obtained for microsatellites in this study was higher than those reported in some studies (Whannou et al., 2023; Yordanov et al., 2024; Cemal et al., 2024) but lower than in others (Kawecka et al., 2022; Lai et al., 2022; Pakpahan et al., 2023; Zhao et al., 2023; Lai et al., 2023; Demiray et al., 2024). The F_{IT} value (0.183) obtained specifically for microsatellites indicates that approximately 18% of the genetic variation in the total population results from excess homozygosity. This value is higher than those reported in some previous studies (Kawecka et al., 2022; Pakpahan et al., 2023; Whannou et al., 2023; Yordanov et al., 2024; Demiray et al., 2024) but lower than the value reported by Cemal et al. (2024). When evaluated collectively, these indices clearly indicate that the primary genetic pattern in the studied Kilis goat populations is not divergence at the provincial level but rather increased homozygosity within populations.

This trend can be attributed to inbreeding within populations rather than genetic differences between populations. It is a natural consequence of the intensive selection processes carried out in the breeding program under the Kilis Goat Breeding Sub-Project. Controlled mating programs and intensive selection pressures implemented during breeding may have reduced genetic diversity. Inbreeding not only increases the risk of homozygosity but can also lead to the emergence of deleterious alleles that negatively affect the breed's overall fitness and adaptability. These findings underscore the need to balance genetic improvement with the preservation of genetic diversity to sustain the contribution of Kilis goat populations to long-term production.

The G_{ST} value (0.011) obtained in this study indicates that most of the genetic variation is attributable to intra-population and intra-individual differences rather than differentiation among populations. This finding reflects a relatively homogeneous genetic structure across the examined populations. Such an outcome aligns with the results of Whannou et al. (2023) and Yordanov et al. (2024), who reported comparable G_{ST} estimates in similar livestock populations. However, the comparatively lower G_{ST} value observed here, relative to those documented in other studies (Pakpahan et al., 2023; Cemal et al., 2024; Demiray et al., 2024), suggests that the populations under investigation exhibit a reduced degree of genetic subdivision. This limited differentiation may be explained by factors such as shared ancestry, gene flow, or common breeding and flock management practices, all of which promote genetic uniformity. This outcome also agrees with the F_{ST} and G_{ST} analyses, collectively demonstrating minimal genetic structuring among the studied groups. The relatively lower diversity values, compared with previous reports (Pakpahan et al., 2023; Cemal et al., 2024; Demiray et al., 2024), may be associated with the geographical proximity of the flocks and their historical breeding management, which likely constrained genetic variability over time. These results highlight the importance of continuous genetic monitoring within livestock populations, particularly within the framework of conservation and breeding programs. While the observed genetic homogeneity may contribute to greater consistency in production traits, it simultaneously underscores the necessity of maintaining sufficient genetic variability to safeguard adaptive capacity and resilience against environmental fluctuations and production-related pressures.

The analysis was conducted within a single breed framework, and the low F_{ST} value (0.022) indicates minimal large-scale inter-population differentiation. Therefore, the observed deviations from Hardy–Weinberg equilibrium are unlikely to be primarily driven by pronounced inter-population divergence. The consistently positive F_{IS} estimates (0.165), calculated using the F-statistics framework of Wright (1990) and the estimators of Weir and Cockerham (1984), reveal a systematic heterozygote deficiency within the population. Given that methodological artifacts such as null alleles were excluded, these patterns reflect intrinsic biological and demographic processes. In field-managed small ruminant populations, the assumption of random mating is rarely fully met. Flock-based management, limited buck exchange, disproportionate use of a few males, and potential related matings can elevate inbreeding coefficients and reduce observed heterozygosity. Additionally, sampling across multiple flocks may introduce micro-level population substructure, producing a Wahlund effect that can generate departures from equilibrium even in the absence of true breed-level differentiation. Although theoretical selection effects could contribute to HWE deviation, the microsatellite loci employed are selectively neutral and not expected to be under direct phenotypic selection. From a breeding program perspective, these findings highlight the importance of structured mating schemes to maintain genetic diversity and minimize further increases in homozygosity.

Implementing systematic buck rotation, monitoring inbreeding coefficients, and ensuring balanced use of males across flocks are essential to preserve heterozygosity and the adaptive potential of the Kilis goat population. The results therefore provide practical guidance for sustainable genetic management, ensuring the long-term viability and productivity of this native breed while mitigating the risks associated with inbreeding and population substructure. Thus, the Hardy–Weinberg deviations observed in this study should be interpreted primarily as consequences of within-population breeding structure and management conditions.

The χ^2 test results for Hardy–Weinberg equilibrium indicated deviations at 14 microsatellite loci in the Gaziantep population and 15 loci in the Kilis population (Table 4). A total of 81 private alleles were identified across the studied populations, with the highest frequency observed in the Gaziantep population. However, most of these alleles—except for two in Gaziantep and five in Kilis—had frequencies lower than 5%. Although these private alleles provide valuable information, their generally low frequencies suggest they have limited discriminatory power for differentiating the populations. Accordingly, these alleles seem to contribute more to within-population allelic richness than to distinct population differentiation. Deviations from Hardy–Weinberg equilibrium observed across most loci can be explained by a combination of population genetic processes and sampling structure. Biologically, the positive FIS values and observed heterozygote deficiency indicate non-random mating and increased relatedness within flocks, consistent with flock-based breeding systems and the use of a limited number of breeding males. Methodologically, potential sources such as genotyping errors or null alleles are unlikely to account for these deviations, as null allele frequencies remained below the accepted threshold. Therefore, the observed deviations from Hardy–Weinberg equilibrium are more plausibly attributed to breeding management practices and within-population structuring—potentially reflecting a Wahlund effect—rather than technical artifacts.

The F_{IS} values of 0.205 and 0.126, along with observed heterozygosity (Ho) values of 0.66 and 0.73 for the Gaziantep and Kilis populations, respectively, indicate that ongoing breeding programs significantly influence the genetic structure of these Kilis goat populations (Table 4). The elevated F_{IS} value in the Gaziantep population reflects a moderate level of inbreeding, likely resulting from mating among related individuals within the breeding program. In contrast, the lower F_{IS} and higher Ho observed in the Kilis population suggest that the breeding strategies implemented there are more effective at maintaining heterozygosity and overall genetic diversity. These differences highlight that, while selective breeding can enhance desirable production traits, careful management is essential to preserve genetic variability. Consequently, these findings underscore the importance of implementing controlled mating schemes and continuous genetic monitoring within breeding programs to ensure the sustainable conservation of genetic resources.

Structure analysis results indicate significant genetic overlap between the Gaziantep and Kilis populations. Additionally, the optimal number of genetic clusters determined using the ΔK method (Evanno et al., 2005) was found to be three, as shown in Table 5. The three clusters identified in the STRUCTURE analysis suggest the presence of distinct genetic subgroups within the population, despite the samples belonging to a single breed collected from two provinces. However, given the low G_{ST} (0.011) and F_{ST} (0.022) values obtained in this study, it is evident that the observed clustering does not reflect true inter-population or inter-breed differentiation but rather low intra-breed genetic structuring. These low differentiation indices indicate limited genetic divergence and substantial gene flow among the sampled provinces, supporting overall genetic homogeneity at the macro-population level. Such intra-breed structuring may result from localized mating patterns, unequal sire contributions, and flock management practices. Importantly, the mean F_{IS} value (0.165) indicates a moderate deficit of heterozygotes within subpopulations, reflecting non-random mating and inbreeding effects. The corresponding F_{IT} value (0.183) further confirms an overall heterozygosity deficit at the total population level. Since STRUCTURE analysis assumes Hardy–Weinberg equilibrium within inferred clusters, heterozygote deficiency caused by inbreeding and/or hidden subpopulation structure can lead to individuals being assigned to separate clusters, even when strong genetic differentiation is absent. This pattern is consistent with the Wahlund effect, in which hidden subpopulation structuring within an apparently homogeneous population leads to heterozygosity deficits and artificial clustering signals (Garnier-Gere and Chikhi, 2013). Therefore, the $\Delta K = 3$ result should be interpreted as evidence of intra-breed subdivision rather than the existence of three genetically distinct populations. Taken together, the low G_{ST} and F_{ST} values, along with moderate F_{IS} and F_{IT} indices, indicate that the Kilis goat population

maintains high genetic similarity across provinces. The observed clustering reflects intra-breed subdivision resulting from relatedness structures, non-random mating, and sampling heterogeneity, rather than true population divergence. These findings suggest that, although large-scale population divergence is minimal, continued monitoring of inbreeding levels is important to prevent the potential long-term erosion of genetic diversity and adaptive capacity.

Recent SNP-based genomic studies on Turkish native goat breeds have demonstrated that population structure and genetic differentiation can be clearly resolved using high-density marker panels. For instance, ddRADseq analyses revealed distinct clustering and measurable genetic divergence among certain local breeds, particularly where breeding isolation and selection history have promoted genetic separation (Bilginer et al., 2025). In contrast, the low F_{ST} (0.022) and G_{ST} (0.011) values observed in the present microsatellite-based study indicate minimal inter-population divergence within the Kilis breed. This difference likely reflects the absence of long-term reproductive isolation and the maintenance of gene flow between provinces. Although SNP markers provide higher genome-wide resolution, the concordance between marker systems in detecting either clear separation or weak differentiation highlights that the observed genetic homogeneity in the Kilis breed represents a biologically meaningful pattern rather than a methodological limitation.

Bottleneck analysis results varied depending on the mutation models applied to the Gaziantep and Kilis populations. Given the current dataset and the types of microsatellites used, the Two-Phase Mutation Model (TPM) combined with the Wilcoxon rank test is considered the most appropriate and reliable approach for assessing population structure. Microsatellite-based bottleneck analyses may produce inconsistent results when mutation processes are modeled solely using the Infinite Allele Model (IAM) or the Stepwise Mutation Model (SMM). Therefore, the Two-Phase Mutation Model (TPM), which incorporates both single-step and multi-step mutations, is generally considered more suitable for detecting heterozygosity excess in microsatellite datasets. In addition, the Wilcoxon signed rank test is regarded as a reliable statistical method for bottleneck detection, particularly in studies analyzing a limited number of loci (fewer than 20) (Di Rienzo et al., 1994; Luikart et al., 1998; Piry et al., 1999). Under the TPM and Wilcoxon test, no significant heterozygote excess was detected in either population, indicating that neither population experienced a severe genetic bottleneck in the recent past. The heterozygote excess signals observed under the IAM model may result from the model's heightened sensitivity to recent demographic changes. In contrast, the absence of significant heterozygote excess in the more conservative SMM model is expected. Therefore, assessments based on the TPM and Wilcoxon tests support the conclusion that Kilis goat populations have not experienced a significant genetic bottleneck in the recent past. However, there is evidence of moderate inbreeding and increased homozygosity within the population, as indicated by the current F_{IS} value (0.165).

These results indicate that genetic diversity has been largely preserved within the populations, while management practices have contributed to partial homozygosity due to limited gene flow. In the context of conservation and breeding programs, strategies such as increasing buck rotation and enhancing gene flow between flocks can be implemented to support genetic diversity and ensure long-term adaptive potential and productivity. Studies conducted on native Turkish goat breeds—such as Kilis, Kil, Angora, Honamli, Norduz, and Kabakulak—similarly report that no bottlenecks have occurred in the populations studied (Ağaoğlu and Ertuğrul, 2012; Demir, 2024; Cemal et al., 2024). Thus, the bottleneck results indicate demographic stability in the recent past. When interpreted alongside the moderate F_{IS} value, the findings suggest that the observed heterozygote deficit is more likely associated with mating structure and breeding management practices rather than a recent reduction in effective population size.

The allele frequency distributions obtained in this study reveal that the genetic structures of Kilis goat populations raised in the provinces of Gaziantep and Kilis are largely similar. The data show that low-frequency alleles predominate in both populations. Specifically, alleles with frequencies in the 0.0–0.1 range constitute approximately 75–80% of the total allele pool, indicating a high prevalence of rare alleles in these populations. This pattern suggests a significant level of genetic variation within the Kilis goat populations, primarily represented by low-frequency alleles. The predominance of rare alleles reflects both a positive indicator of the species' adaptive potential and a potential risk factor due to genetic drift and inbreeding-related losses. Moreover, the near overlap in allele frequency distributions between the Gaziantep and Kilis populations suggests a high degree of genetic

homogeneity, likely resulting from substantial gene flow or a shared genetic history. In this respect, the patterns of allele frequency distribution align with the F-statistics, STRUCTURE analysis, and bottleneck results, collectively supporting the conclusion that Kilis goat populations maintain broad genetic similarity despite detectable within-population structuring.

5. Conclusions

The present study highlights the importance of adopting a comprehensive approach at both intra-population and inter-population levels when developing breeding strategies for the Kilis goat. The findings indicate that the genetic structure of Kilis goat populations is generally homogeneous; however, there is a low level of inbreeding and a tendency toward homozygosity within populations. Bottleneck analyses reveal that the populations studied have not experienced a significant reduction in effective population size in the recent past. While this suggests that current genetic diversity is largely preserved, it emphasizes the need for continuous monitoring of critical parameters such as intra-population homozygosity and limited gene flow. Failure to do so may pose long-term risks to the adaptive capacity and productivity of the Kilis goats. In line with these findings, it is recommended that controlled breeding strategies be implemented to ensure the sustainability and genetic diversity of Kilis goat populations. Specifically, practices such as the rotational use of male animals between populations, increased gene flow among flocks, and balanced mating strategies are essential for reducing inbreeding pressure and preserving genetic diversity. Furthermore, systematic genetic monitoring programs will support both the preservation of adaptive potential and the sustainability of production efficiency by tracking changes in population structure. However, given the sampling scope and the number of microsatellite loci used, these management implications should be regarded as precautionary recommendations rather than definitive breeding guidelines. The current findings primarily highlight potential risk areas and establish a baseline for future, higher-resolution genomic studies and long-term monitoring programs.

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Ethical Statement

Ethical approval for all procedures involving animals was granted by the Animal Experiments Local Ethics Committee of Aydin Adnan Menderes University, with authorization documented under protocol number 64583101/2021/181.

Conflicts of Interest

There is no conflict of interest between the article authors.

Author Contribution Statement

Concept: Gündüz, Z.; Design: Gündüz, Z., Yılmaz, O.; Data Collection or Processing: Gündüz, Z., Ata, N., Keskin, M., Gül, S., Yılmaz Tilki, H.; Statistical Analyses: Yılmaz, O.; Literature Search: Gündüz, Z., Yılmaz, O., Yılmaz Tilki, H.; Writing, Review and Editing: Gündüz, Z., Yılmaz, O., Cemal, İ., Karaca, O.

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