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Microsatellite-Based Insights into Bottleneck Dynamics in Awassi Sheep

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ABSTRACT

Awassi sheep is a strategically important indigenous breed in Türkiye, particularly valued for its dairy performance and environmental adaptability. In this study, 190 individuals sampled from flocks in the Gaziantep and Kilis provinces were genotyped using 17 microsatellite loci to characterize genetic variability and population structure. A total of 306 alleles were detected, indicating substantial allelic richness. The mean observed heterozygosity (Ho = 0.72) was lower than the expected heterozygosity (He = 0.82), suggesting low to moderate heterozygote deficiency potentially attributable to subpopulation structure (Wahlund effect). Genetic differentiation among flock subpopulations was low (F_{ST} = 0.019), indicating ongoing gene flow and weak population subdivision. Bayesian clustering analysis implemented in STRUCTURE identified three genetic clusters independent of strict geographic boundaries. Bottleneck analyses revealed no evidence of a recent reduction in effective population size (Ne). These findings indicate that genetic diversity has been effectively maintained and support the long-term sustainability of the selection scheme conducted under the "National Genetic Breeding Project in Small Ruminants under Breeders' Conditions" initiated in 2005.

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İvesi Koyunlarında Darboğaz Dinamiklerine Mikrosatellit Tabanlı Bakış

ÖZET

İvesi koyunu, Türkiye'de özellikle süt verimi ve adaptasyon kabiliyeti bakımından stratejik öneme sahip yerli bir koyun ırkıdır. Bu çalışmada, Gaziantep ve Kilis illerinde yetiştirilen İvesi sürülerinden örneklenen 190 baş hayvan, 17 mikrosatellit lokusu kullanılarak genotiplendirilmiş ve popülasyonun genetik varyasyon düzeyi ile popülasyon yapısı değerlendirilmiştir. Toplam 306 allel belirlenmiş olup, lokus başına ortalama allel sayısı yüksek allelik zenginliğe işaret etmiştir. Ortalama gözlenen heterozigotluk (Ho = 0,72) değerinin beklenen heterozigotluk (He = 0,82) değerinden düşük olması, popülasyonda kısmi heterozigotluk eksikliği ve olası Wahlund etkisini düşündürmektedir. Sürü alt popülasyonları arasındaki genetik farklılaşma düzeyi oldukça düşük bulunmuş (F_{ST} = 0,019) ve bu sonuç popülasyonlar arası gen akışının sürdüğünü göstermiştir. Bayesian kümeleme analizi (STRUCTURE), coğrafi dağılımdan bağımsız olarak üç genetik alt kümelenmenin varlığını ortaya koymuştur. Bottleneck testleri, yakın dönemde etkili popülasyon büyüklüğünde (Ne) anlamlı bir daralma olmadığını göstermiştir. Bulgular, 2005 yılında başlatılan Halk Elinde Küçükbaş Hayvan Islahı Ülkesel Projesi kapsamında yürütülen seleksiyon programlarının genetik varyasyonu koruyacak şekilde uygulandığını ve popülasyonun uzun dönemli genetik sürdürülebilirliğinin sağlandığını göstermektedir.

Zootekni

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INTRODUCTION

Sheep breeding in Türkiye is widely practiced nationwide, with a predominant focus on meat production. However, the Awassi sheep stand out as a notable exception due to their remarkable milk production capacity (Biçer et al., 2019; Gül et al., 2019; Gündüz, 2014; Gül and Keskin, 2010). This breed is particularly valued for its adaptability to arid and semi-arid regions, as well as its ability to produce high-quality milk that is rich in fat and protein (Ayadi et al., 2024; Biçer et al., 2019; Haile et al., 2017). These characteristics make Awassi sheep an important resource for dairy production, especially in areas where other livestock may struggle to thrive. The Awassi sheep breed is strategically important in sustainable animal husbandry for its resilience to harsh environmental conditions and its significant contribution to the agricultural economy. Selecting livestock with exceptional adaptability and reproductive efficiency is crucial for enhancing productivity, particularly in regions such as Türkiye, where the effects of climate change are becoming increasingly evident (Tüfekçi and Çelik, 2021). Among these, the Awassi sheep stands out as a premier choice for milk production, renowned for its resilience and consistently high yields, even in challenging sub-tropical and arid environments.

The Awassi breed is historically associated with the Fertile Crescent, a region of significant geographical and archaeological importance encompassing present-day Iraq, Syria, Lebanon, Israel, Palestine, Jordan, and southeastern Türkiye. This area is recognized as one of the primary centers of sheep domestication and early agricultural development, dating back approximately 10,000 years (Zeder and Hesse, 2000; Zeder, 2008; Naderi et al., 2008; Pariset et al., 2011). Due to its domestication history, the region is considered an important source of genetic diversity in sheep populations. Preserving this genetic diversity is crucial not only for maintaining breed-specific performance traits but also for conserving rare and potentially valuable alleles that may contribute to future breeding strategies, particularly in the face of increasing climatic and environmental pressures (Sponenberg et al., 2019; Daly et al., 2025). Understanding the genetic variability of Awassi populations is essential for the design of effective conservation and breeding programs (Karaca and Cemal, 1998; Gül et al., 2020; Karaköse and Gündüz, 2023).

Evaluating the genetic structure of Awassi populations in Türkiye provides valuable insights into conserving genetic lineages originating from this historically significant center of domestication. It also contributes to the broader objective of sustaining global small ruminant biodiversity and long-term adaptive potential. Efforts to enhance productivity in farm animals through breeding programs can inadvertently endanger their adaptive traits. Nonetheless, these initiatives highlight the crucial importance of conserving genetic resources to maintain biodiversity and ensure sustainable livestock production (Karaca and Cemal, 1998). As the significance of genetic resource conservation gains global recognition, research in this field continues to expand, including in Türkiye. Achieving a comprehensive understanding of genetic variability is essential for effectively preserving the genetic diversity of domestic animal populations. The National Project for Breeder-Based Small Ruminant Breeding, initiated by the General Directorate of Agricultural Research in 2005, has played a pivotal role in advancing livestock breeding in Türkiye. By implementing customized breeding plans for various breeds and establishing modern breeding organizations, the project has enhanced genetic potential while preserving biodiversity. These efforts have integrated scientific methodologies with practical field applications, promoting sustainable breeding and supporting the adaptation of small ruminants to environmental and production challenges (Karaca et al., 2014).

Microsatellites have emerged as indispensable genetic markers in conservation and breeding programs due to their unique characteristics. These markers are highly polymorphic and multiallelic, providing exceptional levels of genetic variability that make them particularly informative for population studies. Their widespread distribution throughout the genome ensures comprehensive coverage, allowing investigators to analyze a broad range of genetic traits (Gül et al., 2020; Demiray et al., 2024; Cemal et al., 2024). Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are short nucleotide motifs (1–6 base pairs) repeated in tandem and abundantly distributed across eukaryotic genomes (Katti et al., 2001). Their high mutation rate, primarily driven by replication slippage, results in substantial allelic variation even among closely related individuals. This codominant inheritance pattern allows for the detection of both homozygous and heterozygous genotypes, making microsatellites particularly effective for estimating genetic diversity parameters such as allele number, heterozygosity, polymorphism information content (PIC), and inbreeding coefficients. Furthermore, microsatellite data are widely used to infer population structure, gene flow, genetic differentiation (F-statistics), and recent

demographic events, including population bottlenecks (Toth et al., 2000; Katti et al., 2001; Li et al., 2004; Srivastava et al., 2019). These attributes not only enhance the accuracy and efficiency of genetic assessments but also render microsatellites an accessible and cost-effective tool for advancing the preservation of genetic diversity and improving breeding strategies in various agricultural and conservation contexts (Scribner and Pearce, 2000; Vieira et al., 2016).

A population bottleneck refers to a sharp reduction in effective population size caused by intensive selection programs, management practices, environmental factors, or disease outbreaks, resulting in a temporary loss of genetic variation (Bouzat, 2010). Such demographic contractions reduce the effective population size (Ne) and intensify the effects of genetic drift, accelerating the loss of allelic diversity, particularly of rare alleles. Bottlenecks can lead to reduced heterozygosity, loss of alleles (especially rare ones), and increased inbreeding, which may compromise long-term adaptability and fitness (Lande and Barrowclough, 1987; Carson, 1990). Detecting bottleneck signals through tests for heterozygosity excess, allele frequency distribution analyses (mode-shift analysis), and comparisons between observed and expected genetic diversity provides essential information for conservation planning and the sustainable management of livestock populations (Luikart et al., 1998)

This research was focused on exploring the population structure of Awassi sheep flocks, with the overarching goal of establishing a robust framework to inform strategic decision-making in conservation and breeding programs. By analyzing the genetic variability both within and between these flocks, we aimed to provide critical insights into their genetic diversity, adaptability, and potential for improvement. The findings are expected to serve as a foundation for designing effective strategies to preserve genetic resources while optimizing breeding practices, thereby ensuring the long-term sustainability and productivity of Awassi sheep populations.

MATERIAL and METHOD

Ethical approval for this study was obtained from the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (approval no. 64583101/2021/181).

Sample Collection and DNA Extraction

In the present study, sampling was conducted on Awassi sheep populations in accordance with the criteria established by the FAO (2011) as part of the National Project for Breeder-Based Small Ruminant Breeding in the provinces of Gaziantep and Kilis. The study's animal subjects comprised 190 Awassi sheep, with 96 from Gaziantep and 94 from Kilis provinces. Animals were sampled from multiple independent flocks, including seven flocks in Gaziantep and five in Kilis. Within each flock, individuals were randomly selected to minimize sampling bias. This sampling design aimed to ensure that the collected samples adequately represent the genetic structure of the Awassi sheep populations in the regions. All procedures involving animals were carried out in strict accordance with national and institutional animal welfare regulations and international ethical standards for the care and use of animals in research. Blood samples, approximately 9 mL each, were collected from the jugular vein of each animal using minimally invasive techniques and vacuum tubes preloaded with the anticoagulant ethylenediaminetetraacetic acid (EDTA) (BD Vacutainer Systems, Plymouth, UK). White blood cell nuclear DNA was extracted utilizing the high-salt method as described by Montgomery and Sise (1990) and Miller et al. (1988) and was subsequently stored at −20°C for further analysis. Post-extraction, DNA quality and quantity were determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) to ensure its suitability for downstream applications.

PCR and Fragment Analysis

For this study, 17 microsatellite loci were selected in accordance with the FAO (2011) guidelines for the molecular genetic characterization of animal genetic resources. The selection criteria included high polymorphism information content (PIC), clear and reproducible amplification profiles, suitability for multiplex PCR analysis, and distribution across different chromosomes to ensure genome-wide representation and minimize linkage bias. All selected loci have been widely used and validated in sheep genetic studies, with their polymorphism levels, amplification success, and reproducibility previously demonstrated in ovine populations (Alarslan et al., 2021; Ata et al., 2025). Furthermore, the PIC values were high, with nearly all loci ≥ 0.7 and only two loci slightly lower (0.62 and 0.68), indicating that these markers are highly informative for genetic diversity and population structure analyses. The forward primers were tagged with WellRED dyes for identification (D2, D3, or D4) to ensure compatibility with the Beckman Culter GeXP genetic analyzer (Beckman Colter, Inc., USA), thereby facilitating fragment analysis. A detailed compilation of the multiplex groups formed by the microsatellites is shown in Table 1.

Table 1. Microsatellite marker details for fragment analysis in multiplex groups
Çizelge 1. Multipleks gruplarda fragman analizine yönelik mikrosatellit detayları

Multiplex	Dye	Microsatellites	Primer sequences	Allele range (bp)
M1	D2	OarFCB20	F AAATGTGTTTAAGATTCCATACAGTG R GGAAAACCCCCATATATACCTATAC	95-120
	D2	OarAE0129	F AATCCAGTGTGTGAAAGACTAATCCAG R GTAGATCAAGATATAGAATATTTTTCAACACC	133-159
	D2	ILSTS5	F GGAAGCAATGAAATCTATAGCC R TGTTCTGTGAGTTTGTAAGC	174-218
	D3	OARFCB193	F TTCATCTCAGACTGGGATTCAGAAAGGC R GCTTGGAAATAACCCTCCTGCATCCC	96-136
	D3	OarFCB304	F CCCTAGGAGCTTTCAATAAAGAATCGG R CGCTGCTGTCAACTGGGTCAGGG	150-188
	D3	ILSTS011	F GCTTGCTACATGGAAAGTGC R CTAAAATGCAGAGCCCTACC	256-294
	D4	OARCP34	F GCTGAACAATGTGATATGTTCAGG R GGGACAATACTGTCTTAGATGCTGC	112-130
	D4	INRA0132	F AACATTTCAGCTGATGGTGGC R TTCTGTTTTGAGTGGTAAGCTG	152-178
	D4	D5S2	F TACTCGTAGGGCAGGCTGCCTG R GAGACCTCAGGGTTGGTGATCAG	181-211
M2	D2	OARJMP29	F GTATACACGTGGACACCGCTTTGTAC R GAAGTGGCAAGATTCAGAGGGGAAG	96-150
	D2	MAF214	F GGGTGATCTTAGGGAGGTTTTGGAGG R AATGCAGGAGATCTGAGGCAGGGACG	174-282
	D3	McM0527	F GTCCATTGCCTCAAATCAATTC R AAACCACTTGACTACTCCCCAA	165-187
	D3	CSRD0247	F GGACTTGCCAGAACTCTGCAAT R CACTGTGGTTTGTATTAGTCAGG	220-247
	D4	OARFCB128	F ATTAAAGCATCTTCTCTTTATTTCCTCGC R CAGCTGAGCAACTAAGACATACATGCG	96-130
	D4	BM1329	F TTGTTTAGGCAAGTCCAAAGTC R AACACCGCAGCTTCATCC	160-182
	D4	DRBP1	F ATGGTGCAGCAGCAAGGTGAGCA R GGGACTCAGTCTCTCTATCTCTTTG	195-229
	D4	HSC	F CTGCCAATGCAGAGACACAAGA R GTCTGTCTCCTGTCTTGTCATC	267-301

Table 2. Thermal cycler protocols utilizing the Touchdown PCR method
Çizelge 2. Touchdown PCR yöntemi kullanılarak uygulanan termal döngü protokolleri

Multiplex groups	First denaturation	Denaturation	Annealing	Extension	Final extension	Sum of all cycles
M1	95 °C (5 min)	95 °C 40 (sec)	63-54 °C 40 (sec)	72 °C 60 (sec)	72 °C (5 min)	36
M2	95 °C (5 min)	95 °C 40 (sec)	60-50 °C 40 (sec)	72 °C 60 (sec)	72 °C (5 min)	36

The PCR reactions were performed using genomic DNA and carefully optimized concentrations of the necessary reagents. 50 ng of genomic DNA was combined with sterile distilled water to amplify the target primer regions. The PCR reactions were carried out in a 20 µL final volume, containing 1X PCR buffer, 2.0 mM $MgCl_2$, 0.20 mM of a dNTP mixture (dATP, dTTP, dGTP, dCTP), 0.10 µM of each forward and reverse primer, and 1 U of Taq DNA polymerase (Thermo Scientific, USA). DNA amplification was performed using the touchdown PCR protocol, as outlined by Hecker and Roux (1996). The cycling procedure consisted of distinct phases: denaturation, annealing, and extension. Temperature and time parameters were specifically adjusted for each multiplex group, as presented in Table 2. DNA amplification was carried out on a C1000 Touch™ thermal cycler (Bio-Rad, USA). The

fluorescently tagged PCR products were then separated and analyzed using capillary electrophoresis with Beckman GeXP Genetic Analyzer (Beckman Coulter, Inc., USA).

Statistical Analysis

The population structure was evaluated using a variety of statistics, including the number of alleles (Na), mean number of alleles (MNa), effective number of alleles (Ne), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), and Wright's F statistics (F_{IT}, F_{IS}, F_{ST}) (Wright, 1990; Weir and Cockerham, 1984). In addition, the population's adherence to Hardy–Weinberg equilibrium was analyzed, and null allele frequencies (F(null)) were estimated using CERVUS 3.0.3, based on Brookfield's Method 2 (1996). The calculations were performed using GenAlEx (Peakall and Smouse, 2012) and CERVUS 3.0.3 (Marshall, 2006; Kalinowski et al., 2007) software. All 190 individuals were successfully genotyped across all loci, and no missing data were observed; thus, the effective sample size per locus was 190. The genetic differentiation between populations (D_{ST}) and coefficient of gene differentiation (G_{ST}) were calculated with FSTAT 2.9.3 (Goudet, 2001). A Bayesian clustering approach was employed to examine the population structure was applied using the STRUCTURE (Hubisz et al., 2009; Falush et al., 2007; Pritchard et al., 2000). Population structure was inferred using the STRUCTURE software under the admixture model with independent allele frequencies. This model was preferred to avoid assuming prior correlation among populations and to allow unbiased detection of genetic structure. Each run included a burn-in period of 20,000 iterations followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations. A total of 20 independent replicates were performed for each K value. The number of clusters tested ranged from K = 2 to K = 4, based on the number of sampled populations and to prevent overestimation of the number of clusters. The LOCPRIOR model was not used, and clustering was inferred without incorporating prior population information. The results from multiple runs were aligned and visualized using the CLUMPAK software (Kopelman et al., 2015). The most likely number of clusters (K) was evaluated using the STRUCTURE HARVESTER (Earl and vonHoldt, 2012), based on both the ΔK method (Evanno et al., 2005) and the log probability of the data [LnP(K)]. To identify potential genetic bottlenecks within the populations, the dataset was analyzed using Bottleneck 1.2.0.2 (Piry et al., 1999). Three distinct mutation models were utilized in the analysis: the infinite allele model (IAM), the stepwise mutation model (SMM), and the two-phase mutation model (TPM). For the TPM, the default parameter settings recommended by the Bottleneck software were used, which included a 95% proportion of stepwise mutations and a variance of 12 for multi-step changes (Piry et al., 1999). To assess the statistical significance of the results, the Sign, Standardized, and Wilcoxon signed-rank tests were performed, with 1,000 simulations conducted to ensure a reliable analysis (Piry et al., 1999; Luikart et al., 1998).

RESULTS

This study identified 306 distinct alleles across 17 microsatellite loci. A comprehensive overview of the molecular genetic variability statistics is presented in Table 3.

The highest number of alleles and the effective allele number were detected at the ILSTS5 locus (27) and the DRBP1 locus (12.27), respectively. The highest polymorphic information content (PIC) values (0.91) were observed at the BM1329 and DRBP1 loci. The expected heterozygosity (He) ranged from 0.67 for ILSTS011 to 0.92 for DRBP1, while the observed heterozygosity (Ho) varied from 0.55 for D5S2 to 0.97 for OarFCB20. The mean values of F_{IS}, F_{IT}, and F_{ST} were 0.108, 0.125, and 0.019, respectively. Additionally, the average values for D_{ST}, which reflects the genetic variation between populations, and G_{ST}, the gene differentiation coefficient, were 0.012 and 0.014, respectively.

The χ^2 test was employed to assess the Hardy-Weinberg equilibrium (HWE) of the data gathered from the 17 microsatellite loci in this study. The findings revealed that the allele frequencies for all microsatellite markers significantly deviated from HWE. Furthermore, the frequency of null alleles across all studied loci consistently remained below 20%. The presence of null alleles can lead to an overestimation of F_{IS} values by causing heterozygous individuals to be misclassified as homozygotes, thereby reducing observed heterozygosity. In the present study, although some loci exhibited relatively higher null allele frequencies, all values remained below the commonly accepted threshold of 0.20 (Dakin and Avise, 2004). Therefore, while null alleles may have contributed to a slight inflation of the F_{IS} value (0.108), they are unlikely to fully explain the observed heterozygote deficiency. The positive F_{IS} value is more plausibly attributed to biological factors such as non-random mating, increased relatedness within flocks, and potential sub-structuring within the population. Genetic diversity statistics for the populations are presented in Table 4.

Table 3. Microsatellite loci statistics, Wright's F-Statistics, and Hardy-Weinberg Equilibrium analysis
Çizelge 3. Mikrosatellit lokuslarına ait istatistikler, Wright'in F-İstatistikleri ve Hardy-Weinberg analizi

Locus	Na	Ne	Ho	He	PIC	D_{ST}	G_{ST}	F_{IS}*	F_{IT}*	F_{ST}*	F(Null)	HWE	
												Prob.	Sign.
OarFCB20	21	4.28	0.97	0.77	0.74	0.012	0.016	0.130	0.147	0.019	-0.134	0.000	***
OarAE0129	22	8.37	0.83	0.88	0.87	0.018	0.020	0.112	0.128	0.019	0.034	0.000	***
ILSTS5	27	6.19	0.75	0.84	0.83	0.012	0.014	0.108	0.125	0.019	0.063	0.004	**
OarFCB193	18	5.63	0.68	0.82	0.81	0.004	0.004	0.103	0.121	0.020	0.096	0.000	***
OarFCB304	18	5.46	0.64	0.82	0.80	0.014	0.018	0.101	0.118	0.019	0.124	0.000	***
ILSTS011	13	3.00	0.59	0.67	0.62	0.014	0.021	0.108	0.124	0.019	0.056	0.000	***
OarCP34	12	3.39	0.88	0.71	0.68	0.005	0.007	0.127	0.144	0.020	-0.150	0.000	***
INRA0132	17	7.43	0.83	0.87	0.85	0.007	0.008	0.112	0.129	0.020	0.021	0.000	***
D5S2	8	4.21	0.55	0.76	0.73	0.008	0.010	0.097	0.115	0.019	0.165	0.000	***
OARJMP29	21	5.54	0.65	0.82	0.80	0.011	0.014	0.101	0.119	0.019	0.130	0.000	***
MAF214	16	4.63	0.56	0.78	0.75	0.012	0.015	0.097	0.114	0.019	0.177	0.000	***
McM0527	13	5.41	0.65	0.82	0.79	0.001	0.001	0.101	0.119	0.020	0.119	0.000	***
CSRD0247	22	5.54	0.71	0.82	0.80	0.021	0.026	0.107	0.123	0.018	0.078	0.000	***
OarFCB128	19	6.59	0.70	0.85	0.83	0.013	0.016	0.104	0.121	0.019	0.095	0.000	***
BM1329	22	11.37	0.85	0.91	0.91	0.042	0.046	0.113	0.127	0.016	0.033	0.000	***
DRBP1	22	12.27	0.83	0.92	0.91	0.002	0.002	0.108	0.126	0.020	0.053	0.000	***
HSC	15	7.52	0.63	0.87	0.85	0.003	0.003	0.096	0.114	0.020	0.164	0.000	***
Mean	**18**	**6.28**	**0.72**	**0.82**	**0.80**	**0.012**	**0.014**	**0.108**	**0.125**	**0.019**			

Na: Number of alleles, Ne: Effective number of alleles, Ho: Observed heterozygosity, He: Expected heterozygosity, PIC: Polymorphic information content, D_{ST}: The diversity between breeds, 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, Prob.: P value from chi-square test for Hardy–Weinberg equilibrium, **: P<0.01, ***: P<0.001

Table 4. Polymorphism statistics of Awassi sheep populations from different locations
Çizelge 4. Farklı bölgelerdeki İvesi koyun popülasyonlarının istatistikleri

Location	MNA	Mean heterozygosity		F_{IS}	HWE	NPA		
		Ho	He			Freq. ≥5%	Freq. <%5	Total
Gaziantep	15.35	0.72	0.80	0.116	14	1	46	47
Kilis	15.24	0.73	0.81	0.111	16	2	43	45

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

The number of alleles in the Awassi sheep populations from Gaziantep and Kilis was observed to be 15.35 and 15.24, respectively. The observed heterozygosity values for the Gaziantep and Kilis populations were found to be 0.72 and 0.73, respectively. The inbreeding coefficient (F_{IS}), an indicator of within-population heterozygote deficiency, was estimated as 0.116 for the Gaziantep population and 0.111 for the Kilis population. These positive F_{IS} values indicate a measurable deficit of heterozygotes within populations. In sheep populations, F_{IS} values close to zero are generally interpreted as random mating, whereas values around 0.10 or higher suggest increasing levels of heterozygote deficiency that may be associated with non-random mating, population substructure, or selection (e.g., Wahlund effect) (Wright, 1969; Frankham et al., 2010). In this context, the F_{IS} values obtained in the present study can be considered to reflect a low-to-moderate level of inbreeding rather than severe inbreeding. In this study, a total of 92 unique alleles were detected across the two populations, with one allele in the Gaziantep population and two alleles in the Kilis population exhibiting frequencies greater than 5%.

A considerable number of private alleles were detected in both populations; however, most of these alleles occurred at low frequencies (<5%). Only a limited number of private alleles were present at frequencies above 5%, suggesting that most private alleles represent rare variants rather than strong population-specific signatures. Low-frequency private alleles generally have limited power for distinguishing populations, as they are more susceptible to sampling effects and genetic drift. Therefore, although these alleles contribute to within-population genetic diversity, their overall contribution to population differentiation appears to be limited. This interpretation is consistent with the low F_{ST} value (0.019), indicating low genetic differentiation between the studied populations.

The findings from the STRUCTURE analysis, which included multiple cluster numbers (K=2 to K=4), are presented in Figure 1.

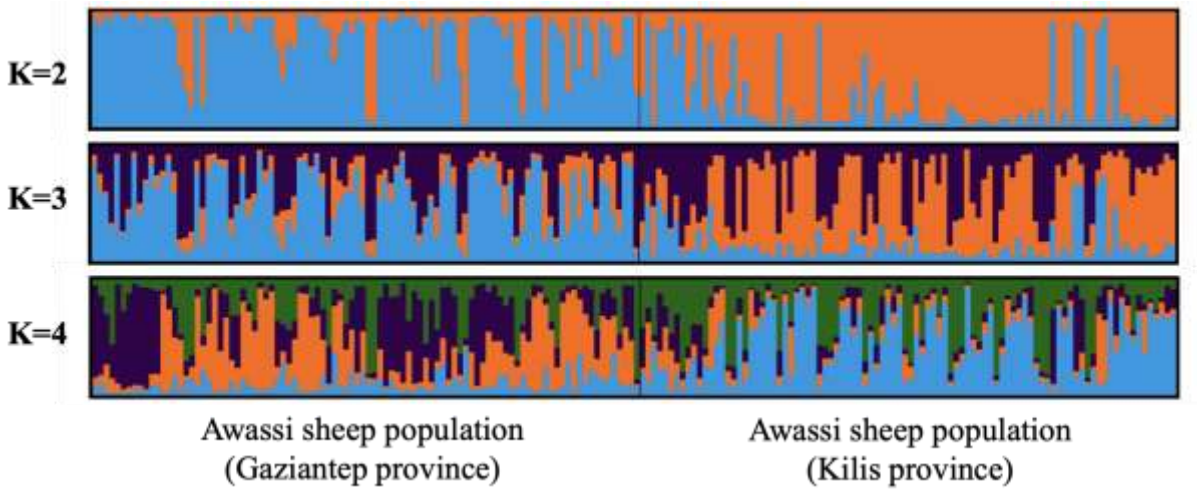


Figure 1. Population structure of Awassi sheep inferred using STRUCTURE analysis and visualized with CLUMPAK for K = 2, 3, and 4.

Şekil 1. STRUCTURE analizi ile belirlenen ve CLUMPAK ile görselleştirilen K = 2, 3 ve 4 için İvesi koyun popülasyonlarının genetik yapısı

Each vertical bar represents an individual and is divided into K colored segments, each corresponding to the individual's estimated membership coefficients for the inferred genetic clusters. The proportion of each color within a bar indicates the probability of assignment to a specific cluster. The colors represent the model-derived genetic clusters and are used solely for visualization; they do not correspond to predefined populations or geographic origins.

The findings, including the posterior probability estimates ([Ln Pr(X|K)]) for the number of clusters (K) and the corresponding ΔK values, are presented in Table 5.

Table 5. Estimated posterior probabilities ([Ln Pr(X|K)]) for different clustering (K) and ΔK values

Çizelge 5. Farklı kümeleme (K) ve ΔK değerleri için tahmini olasılıklar ([Ln Pr(X|K)])

K	Reps	Mean LnP(K)	ΔK
2	20	-12.667,22500	---
3	**20**	**-12.688,11500**	**450.236**
4	20	-12.621,68000	---

The calculated ΔK value, using the method proposed by Evanno et al. (2005), indicates that there are three distinct genetic groups within the studied population. The obtained dataset was analyzed to investigate potential genetic bottlenecks. For this purpose, three different mutation models were utilized, as mentioned by Luikart et al. (1998), and Piry et al. (1999). The analysis utilized the Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two-Phase Mutation Model (TPM). The outcomes of the bottleneck assessment are summarized in Table 6.

To detect potential bottlenecks in the Awassi sheep populations under investigation, a Mod-Shift graph was constructed using allele-frequency categories derived from 17 microsatellite loci. The generated graph is displayed in Figure 2. Allele frequency distribution analysis was performed by grouping alleles into frequency classes, as implemented in the BOTTLENECK software. According to Luikart et al. (1998), populations at mutation–drift equilibrium typically exhibit an L-shaped distribution, characterized by a high proportion of low-frequency alleles. In contrast, populations that have experienced a recent bottleneck exhibit a mode-shift distortion, in which intermediate-frequency alleles become more common than rare alleles due to the rapid loss of low-frequency alleles. In the present study, both populations displayed a normal L-shaped distribution, with the largest proportion of alleles in the lowest-frequency class. This pattern is consistent with mutation–drift equilibrium and provides no evidence of a recent genetic bottleneck.

Luikart et al. (1998) described allele frequency distribution patterns as a useful approach for detecting recent population bottlenecks, with a normal L-shaped distribution indicating mutation–drift equilibrium. In the present study, bottleneck inference was evaluated under IAM, TPM, and SMM mutation models. Although the Wilcoxon test under the IAM model indicated a significant result for the Kilis population (P = 0.0284), this signal was not supported by the TPM or SMM models, under which the Wilcoxon test results were non-significant. Such discrepancies among mutation models are expected, as the Infinite Allele Model (IAM) represents a simplified

mutation process that is generally considered less appropriate for microsatellite data. In contrast, the Two-Phase Mutation Model (TPM) is regarded as more realistic for microsatellite loci, as it incorporates both stepwise and multistep mutations. Furthermore, the allele frequency distribution in both populations exhibited a normal L-shaped pattern, indicating the absence of a recent bottleneck. Therefore, the significant IAM result for the Kilis population should be interpreted cautiously as a model-dependent signal rather than as robust evidence of a recent demographic bottleneck. Overall, the combined evidence from TPM and mode-shift analysis does not support the presence of a recent bottleneck in the studied Awassi sheep populations.

Table 6. Bottleneck analysis in Awassi sheep populations
Çizelge 6. İvesi koyun popülasyonlarında darboğaz analizi

Breed	Mutation Models	Sign test				Standardized differences test		Wilcoxon rank test (one tail for H excess)
		Hee	Hed	He	P	T2	P	P
Gaziantep	IAM	10.29	5.00	12.00	0.2795	1.07	0.1419	0.0727
	TPM	10.01	12.00	5.00	0.0135	-4.99	0.0000	0.9961
	SMM	9.99	17.00	0.00	0.0000	-14.84	0.0000	1.0000
Kilis	IAM	10.31	5.00	12.00	0.2824	1.47	0.0707	0.0284
	TPM	10.05	13.00	4.00	0.0032	-3.76	0.0001	0.9945
	SMM	10.08	15.00	2.00	0.0001	-12.10	0.0000	1.0000

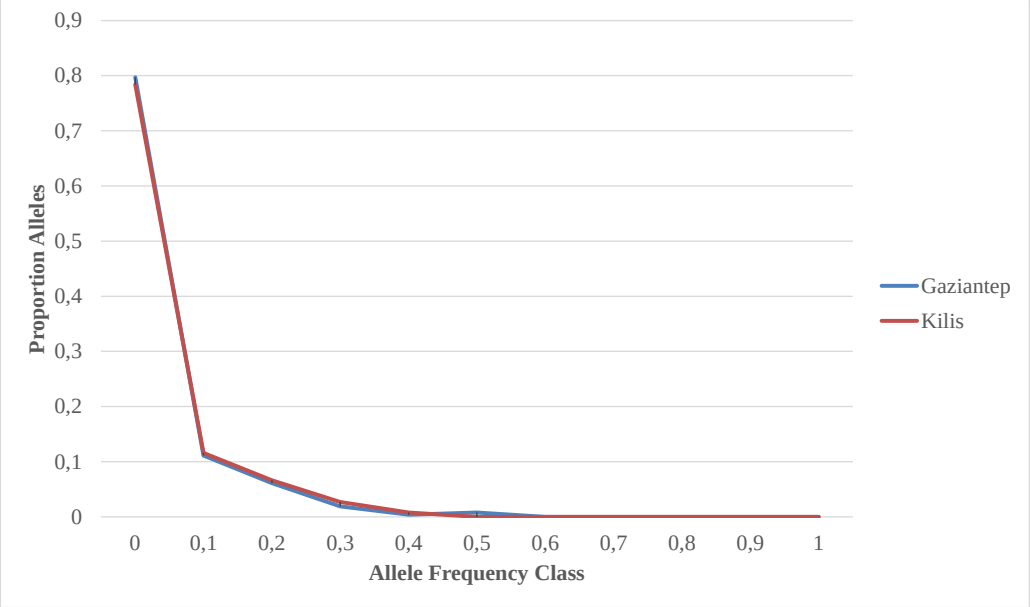


Figure 2. A Mod-shift graph illustrating the genetic bottleneck in the Awassi sheep breed
Şekil 2. İvesi koyunlarında Mod-shift grafiği

DISCUSSION

The mean PIC value obtained in the present study was 0.80, indicating a high level of polymorphism in the analyzed loci. This value exceeds those reported in several previous studies, including Whannou et al. (2022) and Odjakova et al. (2022), which reported PIC values around 0.70–0.73, as well as Mishra et al. (2023), Loukovitis et al. (2023), and Isakova et al. (2023), where values ranged from 0.72 to 0.75. The PIC value observed here is also slightly higher than those reported by Marcovic et al. (2022), Odjakova et al. (2023), and Mihailova et al. (2023), which were close to 0.78–0.79. In contrast, it is lower than the values reported by Suvarna et al. (2023) and especially Kabasakal (2023), who reported PIC values of 0.84 and 0.90, respectively. Such variation among studies may be attributed to differences in breed characteristics, sample size, geographical origin of the populations, and particularly the number and informativeness of the microsatellite loci used. Additionally, the inclusion of more polymorphic loci or the analysis of genetically more heterogeneous populations may increase PIC estimates. Therefore, the relatively high mean PIC value obtained in this study supports both the presence of substantial genetic variability in the Awassi sheep populations examined and the effectiveness of the selected microsatellite marker panel in capturing this variation.

In the present study, the observed heterozygosity (Ho = 0.72) and expected heterozygosity (He = 0.82) indicate a genetically diverse population, although the difference between these values suggests a degree of heterozygote deficiency. Compared to previous studies, the Ho value obtained here is higher than those reported by Karslı (2024; Ho = 0.31), George et al. (2024; Ho = 0.50), Bayraktar (2024; Ho = 0.35), Mishra et al. (2023; Ho = 0.69), Machova et al. (2023; Ho = 0.41–0.42), Suvarna et al. (2023; Ho = 0.10), and Whannou et al. (2022; Ho = 0.68). Conversely, it is lower than the values reported by Deus et al. (2024; Ho = 0.78), Krivoruchko et al. (2024; Ho = 0.80), Kabasakal (2023; Ho = 0.89), Odjakova et al. (2023; Ho = 0.76), Mihailova et al. (2023; Ho = 0.76), Isakova et al. (2023; Ho = 0.73), Marcovic et al. (2022; Ho = 0.73), and Odjakova et al. (2022; Ho = 0.75), while it closely matches the value reported by Loukovitis et al. (2023; Ho = 0.72). For expected heterozygosity, the He value obtained in this study (0.82) is higher than those reported by Karslı (2024; He = 0.29), George et al. (2024; He = 0.65), Bayraktar (2024; He = 0.36), Mishra et al. (2023; He = 0.75), Machova et al. (2023; He = 0.40–0.43), Loukovitis et al. (2023; He = 0.71), Odjakova et al. (2023; He = 0.76), Mihailova et al. (2023; He = 0.78), Isakova et al. (2023; He = 0.76), Marcovic et al. (2022; He = 0.72), Whannou et al. (2022; He = 0.73), and Odjakova et al. (2022; He = 0.77). However, it remains lower than the values reported by Deus et al. (2024; He = 0.89), Kabasakal (2023; He = 0.91), and Suvarna et al. (2023; He = 0.86). These differences among studies are likely associated with variations in breed characteristics, sample size, number and polymorphism level of microsatellite loci, and the geographic and management conditions of the populations analyzed.

Wright's F-statistics (F_{IS}, F_{IT}, F_{ST}) (Wright, 1990; Weir and Cockerham, 1984) play a crucial role in analyzing and understanding genetic diversity, population structure, and evolutionary mechanisms. In the present study, the F_{IS} value of 0.108 indicates a measurable deficit of heterozygotes, suggesting mild to moderate inbreeding or non-random mating within the populations. Compared to previous studies, the F_{IS} value reported here is higher than those observed by Karslı (2024; F_{IS} = −0.047), Loukovitis et al. (2023; F_{IS} = −0.001), Odjakova et al. (2023; F_{IS} = −0.005), Kabasakal (2023; F_{IS} = 0.032), Mishra et al. (2023; F_{IS} = 0.043), Isakova et al. (2023; F_{IS} = 0.040), Mihailova et al. (2023; F_{IS} = 0.034), Whannou et al. (2022; F_{IS} = 0.03), and Odjakova et al. (2022; F_{IS} = 0.033). It is also moderately higher than the value reported by Deus et al. (2024; FIS = 0.085), but lower than those reported by George et al. (2024; F_{IS} = 0.21), Bayraktar (2024; F_{IS} = 0.17), and particularly Suvarna et al. (2023; F_{IS} = 0.878), which represents an extreme level of homozygosity. The F_{IT} value observed in this study (F_{IT} = 0.125) indicates a moderate overall deviation from Hardy–Weinberg equilibrium. This value is higher than those reported by Odjakova et al. (2023; F_{IT} = 0.038), Mihailova et al. (2023; F_{IT} = 0.078), Whannou et al. (2022; F_{IT} = 0.09), and Odjakova et al. (2022; F_{IT} = 0.046), suggesting relatively greater overall homozygosity in the populations examined here. The average F_{ST} value obtained in this study (F_{ST} = 0.019) indicates very low genetic differentiation between populations. This value is lower than those reported by Deus et al. (2024; F_{ST} = 0.053), Mihailova et al. (2023; F_{ST} = 0.046), Odjakova et al. (2023; F_{ST} = 0.042), Whannou et al. (2022; F_{ST} = 0.06), and Bayraktar (2024; F_{ST} = 0.079), suggesting weaker population structuring in the present study. However, it is slightly higher than that reported by Odjakova et al. (2022; F_{ST} = 0.014), indicating a comparable level of low differentiation. These differences among studies may be attributed to variations in breed structure, sample size, the number and polymorphism level of microsatellite loci, and population management practices. Higher F_{IS} values may be associated with non-random mating and flock-level breeding strategies, while differences in F_{ST} values may reflect varying levels of gene flow and geographic structuring among populations. This phenomenon may be related to the implementation of the "National Project for Breeder-Based Small Ruminant Breeding," which likely contributed to preserving genetic diversity in the studied populations.

The G_{ST} value obtained in the present study (G_{ST} = 0.014) indicates that most genetic variation is distributed within individuals rather than between populations, reflecting a very low level of genetic differentiation and a relatively homogeneous population structure. Compared with previous studies, this value is slightly higher than that reported by Odjakova et al. (2022; G_{ST} = 0.008) but lower than that reported by Odjakova et al. (2023; G_{ST} = 0.018). In contrast, substantially higher G_{ST} values have been reported by Whannou et al. (2022; G_{ST} = 0.06), indicating a greater degree of genetic differentiation in those populations. The low G_{ST} value observed in this study aligns with the low F_{ST} estimate, indicating substantial gene flow and a shared genetic background among the analyzed populations. Variations among studies may result from differences in breed composition, geographic separation, breeding strategies, and the number and informativeness of microsatellite loci employed. Specifically, higher G_{ST} values are generally associated with more geographically isolated or genetically structured populations, while lower values, as observed here, suggest weak population subdivision and ongoing gene flow.

The mean genetic diversity value (D_{ST}) obtained in the present study (D_{ST} = 0.012) further supports the results of G_{ST} and F_{ST}, indicating a very low level of genetic differentiation among the analyzed populations. Compared with previous studies, the D_{ST} value reported here is markedly lower than that reported by Bayraktar (2024; D_{ST} = 0.31) and lower than those reported by Odjakova et al. (2023; D_{ST} = 0.081) and Odjakova et al. (2022; D_{ST} = 0.059). This difference suggests that the Awassi sheep populations examined in the present study exhibit a considerably lower

level of inter-population genetic divergence. The low D_{ST} value observed is consistent with the low F_{ST} estimate and indicates that most genetic variation is maintained within populations rather than between them. This pattern is typically associated with populations experiencing ongoing gene flow, shared breeding practices, or geographic proximity. Differences between studies may be attributed to variations in breed composition, sampling design, marker selection, and population management systems. Higher D_{ST} values are often linked to more structured or geographically isolated populations, whereas the low value observed in this study reflects weak population subdivision. These findings highlight the importance of continuous genetic monitoring in livestock populations. Although a relatively homogeneous genetic structure may promote consistency in production traits, maintaining adequate genetic variability is essential to ensure long-term adaptability and resilience to environmental and production challenges.

The χ^2 test results indicate that the allele frequencies of the 17 microsatellite markers examined deviate significantly from the expected Hardy-Weinberg equilibrium. As previously noted, given the breeding practices applied to the populations from which the animal materials were sourced, the deviation of the loci from Hardy-Weinberg equilibrium is an expected outcome. Notably, the detected frequencies of null alleles for all analyzed microsatellites were under 20%, which falls within the acceptable threshold reported by Dakin and Avise (2004). This finding provides compelling evidence that the microsatellites used in this study can serve as a reliable basis for assessing genetic diversity. Deviations from Hardy–Weinberg equilibrium observed across all loci can be attributed to a combination of biological and methodological factors. From a methodological perspective, null alleles may contribute to an apparent deficit of heterozygotes by causing misclassification of heterozygous individuals as homozygotes. However, in the present study, all null allele frequencies remained below the commonly accepted threshold of 0.20 (Dakin and Avise, 2004), indicating that their impact on HWE deviations is likely limited. From a biological perspective, the positive F_{IS} value and the observed heterozygote deficiency suggest non-random mating and increased relatedness within flocks, which are consistent with typical small ruminant breeding systems characterized by limited numbers of breeding males. In addition, sampling across multiple flocks may introduce micro-level population substructure, leading to a Wahlund effect that can cause deviations from equilibrium even in the absence of true population differentiation. Although selection could theoretically influence HWE, the microsatellite loci used in this study are considered selectively neutral and are not expected to be under direct selection pressure. Therefore, the observed HWE deviations are best interpreted as consequences of within-population structuring and breeding management practices rather than technical artifacts or true population divergence.

The deficit of observed heterozygosity relative to expected heterozygosity ($H_o < H_e$), together with positive F_{IS} values, indicates a measurable heterozygote deficiency within the studied populations. This pattern may result from a combination of non-random mating, breeding management practices, and subtle genetic structure. Although the ΔK method identified $K = 3$ as the most likely number of upper levels of genetic structure, this result should not be interpreted as evidence for the presence of three distinct populations. Given that the sampling design included two geographic populations and the F_{ST} value was very low (0.019), the inferred clustering likely reflects low genetic structure and individual-level variation rather than true population subdivision. In such cases, STRUCTURE is known to partition genetic variation into clusters even in the absence of strong population differentiation, particularly under conditions of admixture. Therefore, the additional cluster observed at $K = 3$ is best interpreted as representing subtle genetic heterogeneity within populations rather than biologically meaningful population differentiation. This interpretation is further supported by the high level of admixture observed in the assignment plots and the overall low level of genetic differentiation.

The results of the χ^2 test for conformity to Hardy-Weinberg equilibrium indicated that 14 microsatellite loci in the Gaziantep Awassi sheep population and 16 loci in the Kilis Awassi sheep population were not in equilibrium. These findings can be attributed to the selection programs implemented in these populations. Across the populations examined, 92 distinct alleles were identified, with the highest number recorded in the Gaziantep population. However, the frequencies of these unique alleles—except for one identified in the Gaziantep population and two in the Kilis population—were below 5%. In other words, the discriminatory power of the observed unique alleles for defining these populations is notably limited.

An examination of Figure 1 highlights notable transitions between populations, particularly pronounced for the Awassi sheep populations raised in Gaziantep and Kilis provinces. Additionally, the ΔK value, calculated using the method proposed by Evanno et al. (2005), indicated $K = 3$ as the most likely uppermost level of genetic clustering. Given the structural dynamics of sheep breeding in Türkiye, gene flow among flocks and populations may occur due to breeding practices and animal exchanges within farmer-based systems. These movements, along with shared breeding histories and management practices, likely contribute to the observed genetic clustering patterns. The inferred genetic structure is likely shaped by a combination of breeding management, gene flow, and population substructure, and should be interpreted in light of these interacting factors.

Genetic bottlenecks can occur due to various factors, including disease outbreaks, natural disasters, human interventions, and other events that significantly reduce population size. When such events transpire, the surviving individuals constitute a smaller subset of the original population. In bottleneck assessments utilizing microsatellites, the Two-Phase Mutation Model (TPM) has been identified as the most effective framework for examining heterozygosity excess (Piry et al., 1999; Luikart et al., 1998; Dirienzo et al., 1994). Furthermore, studies have indicated that the Wilcoxon test can be applied with high reliability, even in analyses involving a limited number of loci (fewer than 20) (Piry et al., 1999). Based on the results of the Wilcoxon test, conducted under the TPM model, no evidence of a mode-shift was observed in the allele frequency distribution, which exhibited a typical L-shaped curve (see Figure 2) in the two studied Awassi sheep populations. This pattern suggests that the Awassi sheep population has not experienced a genetic bottleneck, indicating that there has been no recent decline in effective population size. Similarly, no evidence of a genetic bottleneck has been reported for the sheep populations of Karacabey Merino (Kabasakal, 2023), Güney Karaman (Akay et al., 2020), Karakaş, Karayaka, Tuj (Ata et al., 2019), Gökçeada, Chios, Çine Çapari (Yilmaz et al., 2018), Akkaraman, Morkaraman, Kıvırcık, Hemşin, Karayaka, Dağlıç, Norduz, Kangal, Hamdani, Konya Merino, and Türkgeldi (Koban, 2004), Coimbatore (Kumarasamy et al., 2010), and Kilakarsal (Radha et al., 2011). In contrast, the Poonchi sheep population (Azhar et al., 2020) and the Tamil Nadu population (Girish et al., 2009) have both been reported to have experienced a genetic bottleneck.

LIMITATIONS OF THE STUDY

This study has several limitations that should be considered when interpreting the results. First, the sampling was limited to Awassi sheep populations from only two provinces, which may not fully represent the breed's genetic diversity across its entire distribution range. Second, although microsatellite markers are highly polymorphic and informative for population genetics, they do not provide the genome-wide resolution offered by high-throughput genomic approaches. Third, although null allele frequencies were below the commonly accepted threshold, their presence at certain loci may have influenced FIS estimates and contributed to the observed heterozygote deficiency. Additionally, due to the lack of baseline data prior to the project, the direct impact of the National Project for Breeder-Based Small Ruminant Breeding on genetic diversity could not be quantitatively assessed. Finally, the most likely number of genetic clusters (K) was determined using the ΔK method, with the inferred clustering pattern supported by both statistical results and biological context.

CONCLUSION

This study provides valuable insights into the genetic composition, structure, and potential genetic bottlenecks within the Awassi sheep populations of the Gaziantep and Kilis provinces. The findings from the study indicate that the populations exhibit significant genetic diversity. As one of the most important breeds in Türkiye, recognized for its resilience and high production traits, the Awassi breed requires the maintenance of genetic integrity. Structure analysis indicates gene flow between Awassi sheep populations in Gaziantep and Kilis, which significantly contributes to their genetic structure. To ensure the sustainability of genetic diversity, breeding programs should prioritize maintaining an effective population size, controlling inbreeding through appropriate mating strategies, and managing genetic variation at the flock level. Additionally, using genetically diverse breeding males and implementing systematic recording and monitoring systems are recommended to prevent the loss of genetic variation over time. Striking a balance between genetic improvement and conservation efforts will ensure the long-term sustainability and continued economic significance of Awassi sheep, both within Türkiye and globally.

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CONTRIBUTION RATE STATEMENT SUMMARY OF RESEARCHERS

Designed research: Z.G., O.Y.; Molecular analysis and investigation: Z.G. and N.A.; Bioinformatic analysis and methodology: O.Y., Data collection: S.G. and M.K.; Writing-original draft preparation: Z.G. and O.Y., Writing-review and editing: İ.C., O.K. All authors approved the manuscript.

CONFLICT OF INTEREST

The contact author has declared that neither of the authors has any competing interests.

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