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Original scientific article

ANALYSIS OF GENETIC VARIATIONS IN TURKISH DOMESTIC GOOSE POPULATIONS AND CHINESE × EMBDEN CROSSES USING MICROSATELLITE MARKERS

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The study investigates the intra-population and inter-population genetic variations of the domestic goose populations of Türkiye and the commercial Chinese - Embden cross genotypes using microsatellite markers. DNA samples were collected from 110 geese of four different populations. The populations' genetic diversity is assessed using the Ans02, Ans17, Ans25, Aalμ1b, Aph19b, and TTUCG5 microsatellite loci. The highest number of alleles were detected at the Ans25 (28) locus, while the Aph19b (14) locus had the lowest. Wright's F-statistics are calculated separately for each locus without discriminating against the populations. The F_{ST} value varied between 0.038 and 0.105 and had positive values in all loci. The number of migrants (N_m) ranged from 2.12 to 6.34. Structure and principal coordinates analysis indicated that the Native population had distinct characteristics from the other populations. The number of shared alleles among populations is accepted as an indicator of genetic erosion in native goose populations. This study is one of the first reports that exotic breeds genetically polluted Turkish native populations.

Keywords: Diversity, DNA, goose, marker, structure

INTRODUCTION

The adaptability of species to changing conditions depends on their genetic diversity. Three factors determine the effectiveness of a change on the species by modifying its genetic diversity: inbreeding, genetic drift, and selection. Genetic diversity is represented by the

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frequency of intra-population and inter-population genes and is usually assessed using molecular markers. Microsatellite markers are fundamental tools used to measure genetic diversity in farm animals (SIMIANER, 2009).

Microsatellites or single sequence repeats (SSR) are used extensively in low and high-throughput genotyping studies. Identification, characterization and examination of the biological functions of many species populations aim to address some theoretical aspects of microsatellites or simple sequence repeats (SSR) (VIEIRA *et al.*, 2016). Compared to other markers, the main advantages are higher statistical power per locus, high allelic enrichment, and high mutation rate (NEOPHYTOU *et al.*, 2018).

The goose belongs to the Anatidae family, and the Anser genus is known for being among the first domesticated fowl. Some debate about the domestication time; it is hypothesized that domestication was about 3,000 years ago (BUCKLAND, 2002; FARRELL, 2004). Most of the commercially used goose breeds are descendants of Wild Greylag Goose (*Anser anser*) and Wild Swan Goose (*Anser cygnoides*) (FARRELL, 2004). Originating from two common ancestors and an intensive artificial selection process over the years have led to the emergence of the breeds and lines that express significant differences in various phenotypic traits, including size, body weight, feather color, behavior, and physiology in addition to the differences in carcass characteristics, taste and meat quality (LUKASZEWICZ, 2010). Native goose populations have certain advantages: their adaptability to natural breeding habitats, the ability to utilize non-arable areas and resistance to diseases (BOZ *et al.*, 2017; ÖNDER *et al.*, 2017). Furthermore, native breeds can serve as a unique resource for unpredictable future breeding requirements (ROMANOV *et al.*, 1996).

The currently used microsatellite markers for geese were detected in the wild forms of goose breeds such as Greylag Goose (*Anser anser*), Canada Goose (*Branta Canadensis*), Swan Goose (*Anser cygnoides*), White-fronted Goose (*Anser albifrons)* and in the domestic goose breeds such as Chinese, Hungarian, Embden, and Zatorska breeds (WEISS *et al.*, 2008; MINDEK *et al.*, 2014). The systematic assessment of genetic diversity and the conservation of native breeds is an essential foundation for conserving intrinsic genetic traits. Elucidation of the superior but not yet established or investigated qualities of the native breeds and survival of these breeds are only possible through their conservation. Recent developments and attention to efficient selection programs have led to genetic improvements in certain breeds. On the other hand, intensive hybridization studies and uncontrolled mating resulted in fewer native goose breeds and even in the endangerment and extinction of certain breeds (GROENEVELD *et al.*, 2010). The genetic diversity studies on animals are based on investigating genetic relationships. Thus, genetic characterization studies are essential in determining the genetic diversity and identification of the breeds (TAPIO *et al.*, 2005).

The demand for goose meat in Türkiye has increased considerably in recent years. Casually uncontrolled mating of domestic geese with commercial genotypes has become widespread. Three autochthonous populations have been bred in mixed flocks with or without phenotypic grouping. Four populations were sampled to represent the general situation in the country. Chinese - Embden cross-population is commercially breeding goose populations originating from other countries. Turkish white, Turkish multicolor, and Pure Native populations consisted of autochthonous goose populations in Anatolia. These populations have been reared in

Anatolia for centuries. Our study aimed to investigate the genetic diversity and population structure of these four goose populations from Türkiye using microsatellite markers.

MATERIAL AND METHODS

The blood samples from a total of 110 domestic geese belonging to four populations, such as Turkish White (26), Turkish Multicolor (28), Pure Native (30), and Chinese - Embden cross (26), were obtained. The DNA was extracted using the salting-out method (Miller *et al.*, 1988). The isolations were checked by electrophoresis on 1% agarose gel, and the DNA concentrations and qualities were determined using a NanoDrop spectrophotometer (Thermo, USA). Template DNAs for the PCR assays were quantified at 20 ng/µL DNA for each sample.

Six highly polymorphic microsatellite loci (Ans02, Ans25, Ans17, Aalµ1b, Aph19b, and TTUCG5) were used in the study (Weiss *et al.*, 2008). Ans02, Ans25, Ans17, Aalµ1b, and Aph19b loci were designed from the Greylag Goose breed (*Anser anser*), while the TTUCG5 locus was designed from the Canada Goose breed (*Branta canadensis*) (MINDEK *et al.*, 2014).

PCR assays were performed in the T100 Thermal Cycler (Bio-Rad, USA) following touchdown procedures to reduce the bands' stuttering. Amplification of the reactions was carried out in a final volume of 20 µL reaction mixture containing 11 µL of nuclease-free dH_2O, 4 µL of Master mix (5X PCR Master Mix, TURGEN), 4 µL of 20 ng/ µL (4 ng/µL) template genomic DNA, 0.5 µL of each 2.5 µM forward and reverse primers (0.625 µM/µL). Final concentrations were indicated in parentheses. PCR conditions were for initial denaturation at 95°C for 5 min; 35 cycles were performed consisting of three steps: denaturation (95°C for 45 s), annealing (45 – 62°C for 45 s) and extension (72°C for 45 s). The final extension step was performed at 72°C for 5 min. PCR products of 660 samples were separated in vertical polyacrylamide gel electrophoresis system (10%, 29:1 acrylamide/bis-acrylamide, 1 mm gel thickness, 5 h, and 30 min running time, 180 V voltages). Gel scorings of the allele peaks and intensity were determined using the gel documentation system's GeneTools® image analysis software (Syngene, UK).

Polymorphism information content (PIC) values of microsatellite loci were determined using Botstein's equation (BOTSTEIN *et al.*, 1980). The number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), fixation index (F), the F statistics of the populations, Nei's unbiased pairwise genetic distance (*D*), pairwise population F_{ST} values and estimates of the number of migrants (N_m) were determined using the GenAlex v6.5 (PEAKALL and SMOUSE, 2012) and GenePop v4.6 (ROUSSET, 2008). Clustering was assessed based on the Bayesian approach implemented in Structure v2.3.4 (PRITCHARD *et al.*, 2000). Ranging from 1 to 10 different numbers of groups (K) with 10 repetitions for each given value of K was evaluated. For each run, the admixture model was used without the LocPrior option with a burn-in period of 10,000 iterations and a post-burn-in simulation length of 1,000,000. The most probable number of clusters was calculated using Structure Harvester v0.6.94 (EARL and VONHOLDT, 2012), which performs the Evanno method (EVANNO *et al.*, 2005). The Genetix v4.05 (BELKHIR *et al.*, 2019) software was also used for the Principal Coordinates Analysis (PCoA) to show the genetic relationship among populations.

RESULTS

All microsatellite loci were determined to be polymorphic. PIC values for Ans02, Ans25, Ans17, Aalμ1b, Aph19b, and TTUCG5 loci were 0.919, 0.925, 0.917, 0.907, 0.879, and 0.904, respectively. The highest number of alleles was determined at the Ans25 (28) locus, while the Aph19b (14) locus had the lowest alleles. The average number of observed alleles in the Turkish White, Turkish Multicolor, Pure Native populations, and Chinese - Embden cross-population was 37.0, 40.3, 41.8, and 38.5, respectively. A minimum of 5 (Ans17) and a maximum of 17 (Ans25) alleles were detected at the investigated microsatellites in all populations. Figure 1 shows an exemplary polyacrylamide gel image obtained for the Aph19b locus.

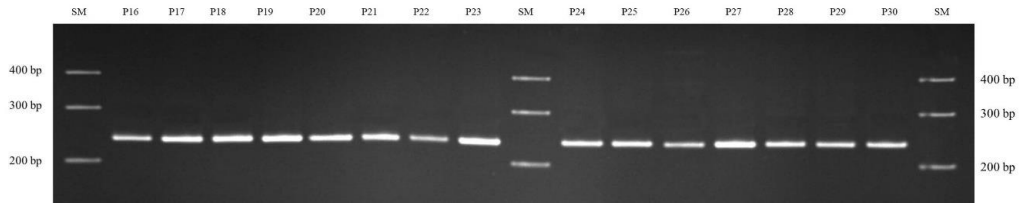

Figure 1. An exemplary polyacrylamide gel image for the Aph19b microsatellite locus

The population genetic parameters used in the present study were summarized in Table 1. The highest degree of genetic diversity was observed in the Turkish Multicolor population (Ne = 8.26 and He = 0.870), and the genetic diversity was lowest in the Pure Native population (Ne = 9.33 and He = 0.800).

Table 1.The sample size (N), the mean number of alleles (Na), the mean number of effective alleles (Ne), the mean observed heterozygosity (Ho), the mean expected heterozygosity (He), and the mean fixation index (F) of all the investigated microsatellite loci of the populations.

Populations	N	N_a	N_e	H_o	H_e	F
Turkish White	26	10.8 (±0.870)	7.31 (±1.15)	0.490 (±0.160)	0.840 (±0.020)	0.430 (±0.190)
Turkish Multicolor	28	12.3 (±1.40)	8.26 (±0.930)	0.520 (±0.160)	0.870 (±0.010)	0.400 (±0.180)
Pure Native	30	9.33 (±1.38)	5.88 (±0.910)	0.470 (±0.180)	0.800 (±0.030)	0.410 (±0.230)
Chinese × Embden	26	11.8 (±1.01)	7.42 (±0.500)	0.550 (±0.150)	0.860 (±0.010)	0.350 (±0.180)

Standard errors of the means are indicated in parentheses.

The examination of the sample size (N), the mean number of alleles (Na), the mean number of effective alleles (Ne), the mean observed heterozygosity (Ho), the mean expected heterozygosity (He), and the mean fixation index (F) values of the populations revealed that the Turkish Multicolor population had the highest value. In contrast, the lowest value was determined in the Pure Native population.

Wright's F-statistics (F_{IS}, FI_T, F_{ST}) were calculated separately for each locus without discriminating against the populations. Table 2 shows Wright's F-statistics calculated at the locus level for Turkish White, Turkish Multicolor, Pure Native breeds, and Chinese - Embden crossbreed.

Table 2. The Wright's F-statistics values at the loci for all populations

Locus	F_{IS}	F_{IT}	F_{ST}	N_m
Ans02	0.525	0.544	0.040	5.96
Ans25	-0.070	-0.030	0.038	6.34
Ans17	0.822	0.841	0.105	2.12
Aalμ1b	-0.214	-0.101	0.093	2.43
Aph19b	1.000	1.000	0.058	4.07
TTUCG5	0.343	0.397	0.082	2.79
Mean	0.401(±0.196)	0.442(±0.183)	0.069(±0.012)	3.957(±0.748)

Standard errors of the means are indicated in parentheses.

The highest F_{IS} and F_{IT} values were observed in locus Aph19b, while the lowest F_{IS} and F_{IT} were recorded for locus Aalμ1b. The F_{ST} values ranged from 0.038 to 0.105 and were positive at all loci. The mean values of F_{IS}, F_{IT}, and F_{ST} were 0.401, 0.442, and 0.069, respectively. According to F_{IS} results, nonrandom mating occurs within each studied population ($F_{IS}>0$). Furthermore, F_{ST} values showed that the degree of genetic differentiation is moderate between the goose populations ($F_{ST}>0.06$). The F_{IT} value was higher than zero, which indicates a deficiency of heterozygosity in the populations. The mean N_m for all loci was found to be 3.957.

The Pairwise Population Matrix of Nei's Unbiased Genetic Distance was shown in Table 3. The highest genetic distance was found between Turkish White and Pure Native (0.946), while the smallest genetic distance was observed between Turkish Multicolor and Pure Native (0.502).

Table 3. Pairwise population matrix of Nei's unbiased genetic distance

	Turkish White	Turkish Multicolor	Pure Native	Chinese × Embden
Turkish White	0.000			
Turkish Multicolor	0.520	0.000		
Pure Native	0.946	0.502	0.000	
Chinese × Embden	0.607	0.698	0.710	0.000

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Pairwise population F_{ST} and N_m values were given in Table 4. Pairwise values of genetic differentiation, F_{ST}, varied from 0.039 to 0.065. Also, N_m values varied from 3.606 to 6.224. Pairwise population F_{ST} was lowest between Turkish White and Turkish Multicolor populations while F_{ST} was highest between Turkish White and Pure Native. Consistently, N_m was lowest between Turkish White and Pure Native, while it was highest between Turkish White and Turkish Multicolor populations.

Table 4. Pairwise population F_{ST} and estimates of Nm values

Populations	F_{ST}	Nm
Turkish White / Turkish Multicolor	0.039	6.224
Turkish White / Pure Native	0.065	3.606
Turkish Multicolor / Pure Native	0.043	5.539
Turkish White / Chinese × Embden	0.043	5.522
Turkish Multicolor / Chinese × Embden	0.042	5.698
Pure Native / Chinese × Embden	0.054	4.422

As a multivariate statistical analysis, the Principle Coordinate Analysis was carried out to determine the genetic variations among the Turkish White, Turkish Multicolor, Pure Native populations, and Chinese - Embden cross-population. The interpopulation relationships were given in Figure 2.

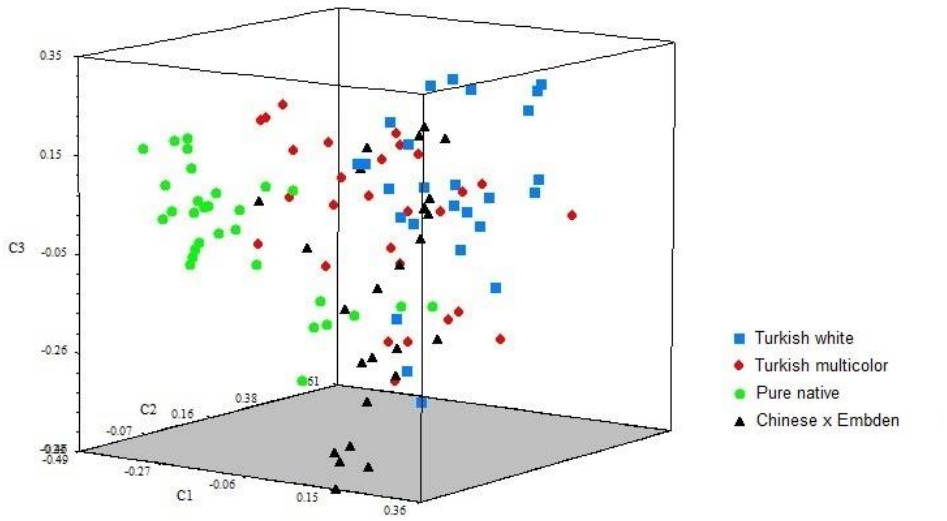

Figure 2. 3D PCoA plots of the populations. Each population was represented by different colors and shapes.

The estimated population structure inferred from all individuals in the populations was shown in Figure 3. The highest ΔK value was determined when K = 2. However, the relatively high Delta K values for K = 3, K=4, and K = 6 indicate that these are also likely options.

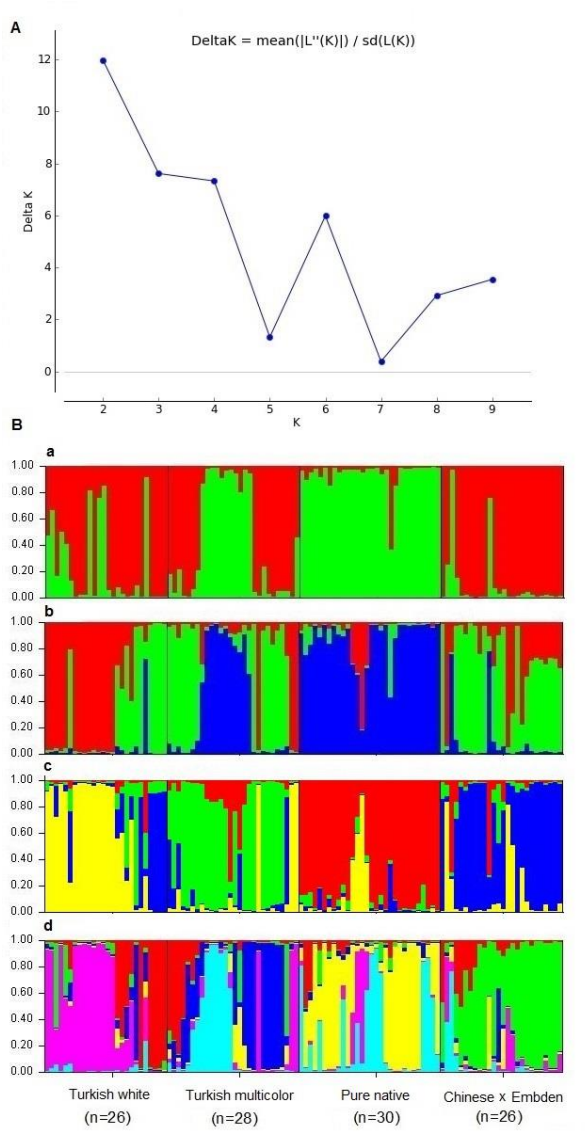

Figure 3. Bayesian cluster analysis results using the Structure Software v2.3.4. (A) DeltaK values generated by Structure Harvester v0.6.94. (B) Estimated population structure; (a) K = 2, (b) K = 3, (c) K = 4 and (d) K = 6. Each individual is represented by a thin vertical line divided into colored segments representing the fraction of the individual's estimated membership of the K clusters.

DISCUSSION

Goose breeding has been traditionally performed in Türkiye for centuries in backyards. Thus, the farming model promises a valuable allelic variety in these populations. Nevertheless, nowadays, they have been progressively abandoned and replaced by exotic breeds, especially Chinese geese. Thus, their extinction or genetic pollution is becoming a real threat.

The PIC values determined in our study were 0.879 to 0.92. Therefore, PIC data provided more informative knowledge about Turkish White, Turkish Multicolor, Pure Native, and Chinese × Embden populations' genetic diversity from similar studies (CATHEY *et al.*, 1998; ABDEL *et al.*, 2019; MINDEK *et al.*, 2019). Instead of increasing the number of monomorphic marker loci, focusing on markers with high polymorphism may yield more accurate results.

The average number of observed alleles were ranging from 37.0 to 41.8. These results were higher than those obtained from the other studies on domestic goose populations (WEISS *et al.*, 2008; ANDRES and KAPKOWSA, 2011; MINDEK *et al.*, 2019). These results show that the allelic richness of the studied populations is very high.

The highest degree of genetic diversity was observed in the Turkish Multicolor population (Ne = 8.26 and He = 0.870), and it was lowest in the Pure Native population (Ne = 9.33 and He = 0.800). Even the Pure Native population's number of effective alleles and expected heterozygosity values are higher than the different originated domestic goose populations (ABDEL *et al.*, 2019 and MINDEK *et al.*, 2019). The observed heterozygosity values determined in this study were ranged from 0.470 in Pure Native to 0.550 in the Chinese - Embden cross population.

The highest mean observed heterozygosity (0.550) and the mean expected heterozygosity (0.870) were determined in the Turkish Multicolor population, while the Pure Native population had the lowest observed heterozygosity (0.470) and expected heterozygosity (0.800). This situation shows a massive deficit of heterozygotes for all populations. The mean allele number and mean observed heterozygosity values found in the present study were close to those reported by MINDEK *et al.* (2014). In the present study, examining the observed and expected heterozygosity values for the loci or the populations revealed that the heterozygosity values were not dissimilar, and heterozygosity was observed in the populations. Negative F_{IS} values at two loci Ans25 (-0.070) and Aalμ1b (-0.214) reveal a heterozygosity excess based on the F statistics, indicating deviations from the Hardy-Weinberg equilibrium and abandonment of pure-breeding. In contrast, positive F_{IS} values indicate that individuals in the populations are more related than expected under a random mating model. Furthermore, F_{ST} values indicated a moderate differentiation between the populations.

The F_{ST} value is the measure of the genetic differentiation among populations. This study's mean F_{ST} value was 0.069 and agreed with other studies (TALBOT *et al.*, 2003; LI *et al.*, 2012; MINDEK *et al.*, 2014). A high F_{ST} value is associated with high interpopulation differentiation. At the same time, a low F_{ST} was attributed to carrying out the rearing activities mostly in small farms, populations' countermovement against differentiation-promoting random genetic drift, and the hindrance of genetic exchange between native goose breeds by the large geographical distances between certain populations (LI *et al.*, 2012). The results indicate that there was a moderate genetic differentiation among the populations.

The farthest populations were Turkish White and Pure Native, according to Nei's Unbiased Pairwise Population Genetic Distance. Pairwise population F_{ST} and estimates of N_m values were consistent with the populations' genetic relationships.

The gene flow value is defined as the individuals migrating between populations per generation, and the N_m value determined in the study varied between 2.12 and 6.34 for all loci. If the number of migrants is less than 1 ($Nm < 1$), genetic drift could significantly influence genetic differentiation between populations and decrease genetic variation. When $N_m \geq 4$, the homogenizing effect of gene flow was sufficient to prevent allele frequencies' stochastic differentiation (KIMURA and WEISS, 1964). Also, LI *et al.* (2011) pointed out that N_m value below 0.500 was an indicator of the predominant genetic drift role in the genetic differentiation in a population. The results revealed that gene flow played a predominant role in the genetic differentiation of the goose populations.

The PCoA results revealed that most of the Pure Native population separated distinctly from the other investigated populations, while White, Multicolor, and Chinese - Embden cross-population were nearer to each other.

Structure analysis-based bar plots have similar patterns that support PCoA results in different K values. The Pure Native population is slightly distinguished from the others in each determined K level.

CONCLUSIONS

This work represents the first attempt to describe indigenous and exotic domestic populations' genetic diversity and structure. Therefore, the study's findings contribute to the genetic comparison of Türkiye's populations to all countries. The study's initial findings revealed that the populations had a rich allelic diversity. Thus, the findings suggest sufficient allelic richness to establish breeding strategies. The Structure and Principle Coordinate Analysis (PCoA) results revealed that the Pure Native population had significant and distinctly different characteristics from those of the other populations. The findings suggest that exotic breeds genetically polluted Turkish native populations.

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ANALIZA GENETSKIH VARIJACIJA KOD TURSKIH DOMAĆIH POPULACIJA GUSAKA I UKRŠTANJA KINESKA × EMBDEN KORIŠĆENJEM MIKROSATELITSKIH MARKERA

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Izvod

Studija istražuje intra-populacijske i međupopulacijske genetske varijacije domaćih populacija guske u Turskoj i komercijalnih genotipova nastalih ukrštanjem kineska x Embden korišćnjem mikrosatelitskih markera. Uzorci DNK su prikupljeni od 110 gusaka iz četiri različite populacije. Genetski diverzitet populacija je procenjen korišćenjem mikrosatelitskih lokusa Ans02, Ans17, Ans25, Aal□1b, Aph19b i TTUCG5. Najveći broj alela otkriven je na Ans25 (28) lokusu, dok je Aph19b (14) lokus imao najmanji broj. Rajtova F-statistika se izračunava odvojeno za svaki lokus bez diskriminacije prema populaciji. Vrednost FST je varirala između 0,038 i 0,105 i imala je pozitivne vrednosti u svim lokusima. Nm kretao se od 2,12 do 6,34. Analiza strukture i glavnih koordinata pokazala je da lokalne populacije imaju različite karakteristike od ostalih populacija. Broj zajedničkih alela među populacijama je prihvaćen kao pokazatelj genetske erozije u autohtonim populacijama guske. Ovastudija je jedan od prvih izveštaja da egzotične rase genetski "zagađuju" turske autohtone populacije.

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