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Assessment of genetic diversity and conservation priorities in some Turkish indigenous Hair goat populations by microsatellite loci

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ABSTRACT

Genetic diversity in livestock breeds is required for breeding studies, response to environmental changes and sustainable production. The aim of this study was to evaluate the genetic diversity in Hair goats reared in 9 districts of Antalya province and to determine the populations that have the highest contribution to the total genetic diversity. For this purpose, 180 samples from 9 districts (Korkuteli-KRK, Elmalý-ELM, Kaþ-KAS, Demre-DMR, Manavgat-MNG, Gündoðmuþ-GND, Ýbradý-IBR, Akseki-AKS and Gazipaþa-GZP) of Antalya province were genotyped by 20 microsatellite loci. The mean number of alleles per locus for each population ranged from 8.45 (GND) to 9.25 (MNG), while mean number of effective allele varied between 5.40 (GND) and 6.22 (MNG). The lowest average observed heterozygosity was in the ELM populations (0.71) while the highest Ho value detected in KAS populations (0.78). Mean expected heterozygosity values varied from 0.80 (GND) to 0.84 (DMR, MNG). Mean PIC values ranged from 0.77 (GND, AKS) to 0.80 (DMR, MNG) in populations. Inbreeding coefficients were detected between 0.05 (KAS) and 0.13 (ELM) in district populations. According to two different methods, the highest contribution to the total genetic diversity comes from KAS (-0.244) and AKS populations (0.482). In conclusion, high genetic diversity and low level of inbreeding were determined in Turkish indigenous Hair goats. Hair goats have great potential for breeding studies and for adaptation to the environmental conditions that will possibly change in the future. Especially, genetic variation in KAS and AKS populations should be conserved.

Keywords: Conservation, Genetic diversity, Hair goats, Microsatellite, Population structure

Goat (*Capra hircus*) is one of the first domesticated animals and has been an important livestock species for humans since domestication (Zeder 2008). Goat breeding is preferred by farmers for many reasons such as, it can be raised on mountainous and rougged lands where agricultural lands are limited, resistance to some diseases and harsh environment conditions and capability to survive under low input systems (Gama and Bressan 2011, Daþkýran *et al*. 2018). Goat breeding is mostly done by small farmers in areas unsuitable for crop production with some exceptions in Turkey. Goats have the important economical contribution to rural economies in developing countries like Turkey (Daþkýran *et al*. 2018). Turkey native goat breeds consist mainly of Hair, Kilis, Angora, Honamlýand Norduz goats. According to official data of 2017, the goat populations in Turkey is approximately 10.5 million of which more than 90% consists of Hair goats (Yýlmaz *et al*. 2012, TUIK 2017a). Goat breeding is very intensive especially in the mountainous areas of Antalya province due to geographical structure, climatic conditions and socio-economic situation. There are approximately 800,000 goats in Antalya province and most of them are Hair goats (TUIK 2017b).

Maintaining genetic variation in livestock breeds is required for breeding studies, meeting the current production levels and adaptation to the environmental conditions that may possibly change in the future. Determination of genetic diversity in livestock breeds is important for conservation and breeding studies (Mahmoudi *et al*. 2010, Lenstra *et al*. 2012, Karslýand Balcýoðlu 2019). Microsatellite markers are extremely useful for the identification of genetic diversity since its high rate of polymorphism and repeatability, random distribution across the eukaryotic genome (in introns and exons regions) and showing co-dominant inheritance. Microsatellite markers are also used to determination of conservation priorities in livestock populations (Iamartino *et al.* 2005, Tadano *et al.* 2013, Karslýand Balcýoðlu 2019).

It is thought that there is a high genetic variation in the hair goats reared in Antalya and significant contribution to total genetic diversity of Turkish Hair goats. The aim of present study was to evaluate the genetic diversity with 20 microsatellite loci in Hair goats reared in 9 districts of Antalya and to determine the populations that are contributing highly to genetic diversity to obtain the basic information for future conservation and breeding studies.

MATERIAL AND METHODS

Sample collection and DNA extraction: Blood samples (approximately 5 ml) were taken into tubes with K3 EDTA

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from 180 goats including 20 samples each from 9 different districts in Antalya province. Blood samples were collected from Korkuteli (KRK), Elmalý(ELM), Kaþ (KAS), Demre (DMR), Manavgat (MNG), Gündoðmuþ (GND), Ýbradý (IBR), Akseki (AKS) and Gazipaþa (GZP) districts. Blood samples were collected from at least three different farms in each district. While collecting blood samples, districts which had less goat population were ignored. The blood samples were stored at –20°C until DNA extraction. Genomic DNA extraction was conducted according to protocol reported by Miller *et al*. (1988) by minor optimization in our laboratory conditions. This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Turkey (Certificate No. 2016.12.01).

PCR and microsatellite genotyping: Twenty microsatellite loci were used recommended by FAO (2011) for the determination of the genetic diversity among Hair goat populations. The PCR reaction mixture consisted of 3 µl genomic DNA (50 ng/µl); 3 µl 10× PCR buffer (75 mM Tris-HCl, *p*H 9.0); 0.3 µl each primer (10 pmol/µl); 0.1 µl Taq DNA polymerase (5 U/µl); 2.5 µl $MgCl_2$ (2.5 mM/ µl) and 3 µl dNTPs (2.5 mM/ µl), and the volume of the mixtures were increased to 25 µL with deionized water. PCR amplification was carried out as follows; initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing (between 50–60°C) for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 10 min. In the present study, 96 automated capillary electrophoresis system (Advanced Analytical Technologies-AATI, Ames, Iowa, USA) was used to determine the fragment size of PCR products. After capillary electrophoresis, each fragment size was determined by using PROSize® 2.0 version 1.3.1.1 (AATI, Ames, Iowa, USA).

Data analyses: The private alleles, allele ranges and frequencies were determined with Convert version 1.31 (Glaubitz 2004) program. Genetic variation parameters (number of allele and effective alleles, observed and expected heterozygosity) were calculated using the Popgene (Yeh *et al.* 1997). Microsatellite Toolkit (Park 2001) for Excel was used to calculate Polymorphic Information Content (PIC) values. Inbreeding coefficient value (F_{IS}) was calculated using Fstat v.1.2 (Goudet 1995). Relationship between goat populations was showed with Factorial Correspondence Analysis (FCA) using Genetix v. 4.05 software (Belkhir *et al*. 2004). Evaluation of the conservation priorities of goat populations according to the method defined by Petit *et al*. (1998) and Caballero and Toro (2002) was carried out using the Moolkin program (Gutierrez *et al*. 2005).

RESULTS AND DISCUSSION

A total of 265 alleles were detected across 20 microsatellite loci. All loci were found as polymorphic with the number of alleles between 6 (ETH10) and 21 (P19, ILTS005). The number of alleles, the effective number of alleles, the observed and expected heterozygosities (Ho and He) and PIC values per each microsatellite locus are shown in Table 1. Effective allele numbers ranged between 3.02 (ETH10) and 12.71 (P19) with the mean of 7.46. The Ho values varied from 0.57 (ETH10) to 0.86 (ILTS087) whereas the He values ranged from 0.67 (ETH10) to 0.92 (P19). The average Ho and He values were 0.74 and 0.84 respectively.

Table 1. Genetic diversity parameters, PIC values and inbreeding coefficients (F_{IS}) obtained per locus in all populations

Locus	N	AR	Na	Ne	H_O	H_E	PIC	F_{IS}
SRCRSP05	165	154–178	13.00	9.29	0.73	0.90	0.88	0.18**
ILTS011	174	218–292	14.00	6.07	0.80	0.84	0.82	0.04
OarFCB48	175	141–165	13.00	7.84	0.73	0.83	0.86	0.12*
SRCRSP03	173	120–136	9.00	3.52	0.58	0.72	0.67	0.19**
SRCRSP07	162	121–137	9.00	5.82	0.82	0.83	0.81	0.01
INRA063	170	168–180	7.00	3.91	0.71	0.75	0.70	0.05
TCRVB6	174	219–259	19.00	10.49	0.72	0.91	0.90	0.21**
MAF70	174	138–168	15.00	6.50	0.65	0.80	0.83	0.18**
ETH10	174	200–210	6.00	3.02	0.57	0.67	0.61	0.12*
SRCRSP09	171	103–135	12.00	7.19	0.84	0.86	0.85	0.03
P19 (DYA)	173	170–220	21.00	12.71	0.84	0.92	0.92	0.08
INRABERN172	170	236–258	12.00	8.14	0.85	0.88	0.86	0.03
MAF65	168	116–148	16.00	7.84	0.78	0.88	0.86	0.11*
SRCRSP15	178	168–194	14.00	8.93	0.73	0.85	0.88	0.14*
MCM527	172	141–167	14.00	7.50	0.83	0.87	0.85	0.05*
CRSD247	160	217–259	15.00	11.04	0.74	0.91	0.90	0.18**
OarAE54	141	113–139	13.00	7.23	0.72	0.86	0.85	0.17**
ILTS005	147	164–218	21.00	7.61	0.60	0.80	0.86	0.21**
SPS113	168	140–162	11.00	8.01	0.83	0.88	0.86	0.05
ILTS087	175	135–155	11.00	6.58	0.86	0.85	0.83	-0.01
Mean			13.25	7.46	0.74±0.09	0.84±0.06	0.83±0.08	0.11*

AR, Allele range; Na, Observed number of allele; Ne, Effective number of allele; H_O, Observed heterozgosity; H_E, Expected heterozgosity; PIC, Polymorphic information content; F_{IS}, Inbreeding coefficient. (*P<0.05; **P<0.01 deviation from Hardy-Weinberg equilibrium).

The mean number of alleles per locus obtained from 20 microsatellite loci (13.25) was lower than that per locus reported (15.65) by Korkmaz Aðaoðlu and Ertuðrul (2012) in 5 different goat breeds reared Turkey for 20 microsatellite loci. However, obtained value in this study were higher than the number of alleles (9.09) per locus reported by Bulut *et al*. (2016) in Hair goats for 11 microsatellite loci. The average Ho value (0.74) in 20 loci was lower than the Ho value (0.78) reported by Korkmaz Aðaoðlu and Ertuðrul (2012) but higher than the Ho value (0.69) reported by Bulut *et al*. (2016).

Basic genetic diversity parameters reported by Korkmaz Aðaoðlu and Ertuðrul (2012) were higher than those obtained in our study. This situation may result from use of 5 different breeds in their study. Genetic differences between breeds can increase number of alleles. Whereas, in the present study, obtained genetic diversity parameters were higher than the values reported by Bulut *et al*. (2016) in Hair goats. It may be due to the high number of sample and locus used in our study. Observed heterozygosity was lower than the expected, because of a deviation from Hardy–Weinberg equilibrium and probability of inbreeding.

Observed heterozygosity was lower than the expected, because of a deviation from Hardy–Weinberg equilibrium (HWE) and estimated inbreeding coefficients (F_{IS}) (mean F_{IS} was calculated 0.11 in twenty microsatellite loci). The average PIC value was estimated as 0.83 for 20 microsatellite loci. This value was higher than values reported in the literature (Ramamoorthi *et al*. 2009, Mahmoudi *et al*. 2010, Korkmaz and Ertuðrul 2012, Tefiel *et al*. 2018). The PIC value is desired to be greater than 0.50 in genetic variation studies. The mean PIC value was 0.83 and this value indicates that the selected microsatellite loci are quite informative for genetic diversity.

The number of alleles, the effective number of alleles, the observed and expected heterozgosities (Ho and He) and PIC values, number of private alleles and F_{IS} values per each population are summarized in Table 2. The mean number of alleles per locus ranged from 8.45 (GND) to 9.25 (MNG), while mean number of effective alleles varied between 5.40 (GND) and 6.22 (MNG). The lowest average observed heterozygosity was in the ELM populations (0.71) while the highest Ho value was detected in KAS populations (0.78). Mean expected heterozygosity values varied from 0.80 (GND) and 0.84 (DMR, MNG). Mean PIC values ranged from 0.77 with 0.80 in populations. The number of private alleles varied from 2 (AKS, KAS) to 5 (ELM). Inbreeding coefficients were detected between 0.05 (KAS) and 0.13 (ELM).

The mean of number of alleles (8.45–9.25) and effective allele (5.40–6.22) per locus obtained from nine Hair goat populations were higher than the Na value (6.33) obtained by Ramamoorthi *et al*. (2009) in Barbari goats from India; Na (5.2) and Ne value (4.12) obtained by Asroush *et al.* (2018) in Markhoz goat breed fom Iran; Na (6.46–8.08) and Ne value (4.17–5.26) obtained by Mahmoudi *et al*. (2010) in three different breeds from Iran; Na value (4.9–8.3) obtained by Iamartino *et al*. (2005) in eight different breeds from Southern Italy. Contrary to the above studies, our results are lower than the Na value (12.91–16.39) reported by Tefiel *et al.* (2018) in four Algerian goat breeds, and in three Kilis goat populations (10.33–11.33) reported by Gürler and Bozkaya (2013). Na values obtained in the present study are similar to Na value (9.09) reported by Bulut *et al*. (2016) in Hair goat population.

The Ho (between 0.71–0.78) and He values (0.80–0.84) obtained were higher than Ho value (0.69) reported by Bulut *et al*. (2016) in Hair goat population, Ho values (0.55–0.62) in nine China native goat breeds and Ho values (0.64–0.66) in three Iran goat breeds reported by Di *et al*. (2011). Otherwise, Ho and He values were lower than Ho value (between 0.80 and 0.89) reported by Gürler and Bozkaya (2013) in three Kilis populations, Ho values (0.82–0.89) reported by Tefiel *et al*. (2018) in four Algerian goat breeds. When the basic genetic diversity parameters were examined in populations, it was seen that there are high genetic diversity in the Hair goat populations reared in Antalya. The reasons of this high genetic diversity in Hair goats raised in Antalya province may be the absence of the systematic selection programs in these populations and

Table 2. Genetic diversity parameters, PIC values, private alleles and inbreeding coefficients (F_{IS}) obtained for 20 microsatellite loci in districts

	n	MNa±SD	MNe±SD	H_O±SD	H_E±SD	PIC±SD	PA	F_{IS}
KRK	20	9.15±2.78	6.03±1.78	0.75±0.14	0.83±0.07	0.79±0.07	3	0.10*
ELM	20	8.70±2.26	5.88±1.66	0.71±0.15	0.83±0.08	0.78±0.09	5	0.13*
KAS	20	8.95±2.46	5.75±1.60	0.78±0.16	0.82±0.07	0.78±0.08	2	0.05
DMR	20	8.80±2.69	6.18±2.09	0.77±0.14	0.84±0.08	0.80±0.09	4	0.08
MNG	20	9.25±2.77	6.22±2.56	0.74±0.19	0.84±0.07	0.80±0.10	3	0.12*
GND	20	8.45±2.48	5.40±1.52	0.75±0.13	0.80±0.06	0.77±0.07	4	0.07
IBR	20	9.20±2.87	5.85±1.71	0.73±0.12	0.83±0.07	0.79±0.09	4	0.11*
AKS	20	8.60±2.60	5.76±2.12	0.73±0.10	0.82±0.09	0.77±0.10	2	0.11*
GZP	20	9.05±2.70	6.18±2.18	0.72±0.12	0.83±0.10	0.79±0.11	3	0.12*

MNa, Mean number of observed allele; MNe, Mean number of effective allele; H_O, Observed heterozgosity; H_E, Expected heterozgosity; PIC, Polymorphism information content; PA, Private allele; F_{IS}, Inbreeding coefficient (*P<0.05; deviation from Hardy-Weinberg equilibrium).

populations are not bred as closed herds (there is breeding material exchange between population).

Number of private alleles (2–5 in 9 Hair goat populations) determined in our study were lower than those found in most studies in different goat breeds (Bosman *et al*. 2015, El Moutchou *et al*. 2017, Tefiel *et al*. 2018). These results are not surprising, as private alleles are associated with migrating individuals between populations. If there is no gene flow between populations, genetic differentiation and the number of private alleles are increasing. However, in our study, there is probably high gene flow between populations because of breeding material exchange by farmers.

The F_{IS} values in this study ranged from 0.05 to 0.13. These values are similar to F_{IS} values (0.03–0.11) reported by Traore *et al*. (2009) in five native goat breeds from Burkina Faso, and F_{IS} in three Kilis goat populations values (0.03–0.08) reported by Tefiel *et al*. (2018) in four Algerian goat breeds. These values indicate that low level of inbreeding in populations. Simon and Buchenauer (1993) reported that breeds are not in serious danger if the F_{IS} value is between 0.05–0.15. There is deviation from Hardy-Weinberg equilibrium due to the heterozygote deficits in six goat populations (KRK, ELM, MNG, IBR, AKS, GZP).

The contribution of each population to genetic diversity based on the methods defined by Caballero and Toro and Petit *et al.* is given in Table 3. According to the method by Caballero and Toro, IBR population has the lowest contribution (0.143) to the genetic diversity among populations. The highest contribution to the total genetic diversity comes from KAS population (-0.244). According to the method developed by Petit *et al.* (1998), the highest contribution to the total genetic diversity was obtained from AKS population (0.482).

According to Caballero and Toro (2002), contribution of each subpopulation to total genetic diversity can be determined when each of the subpopulations is removed from the overall data set. When each subpopulation is removed from the data set, the negative value (-) that occur in the overall data set is the contribution of that population to total genetic diversity. In summary, the subpopulation which has the highest negative value contributes to the total genetic diversity the most. In contrast, Petit *et al.* (1998) reported that the highest positive value contributes to the total genetic diversity the most.

In the present study, according to the method by Caballero and Toro (2002), highest contribution to the total genetic diversity comes from KAS population (–0.244) and lowest from IBR population (0.143). On the contrary, according to the method developed by Petit *et al*. (1998), the highest contribution to total the genetic diversity comes from AKS population (0.482). Similarly to method by Caballero and Toro (2002), lowest contribution to genetic diversity comes from IBR population (-0.199). Different results about highest contribution to genetic diversity may result from methodological differences between two different methods. These methods estimate the contribution of each subpopulation in terms of maximization of gene diversity (Caballero and Toro 2002) or allelic richness (Petit *et al*. 1998). Considering the genetic diversity parameters, inbreeding coefficients and contributions to genetic diversity together, conservation of genetic diversity in the KAS population is thought to be more important.

Table 3. Contributions of goat populations in each district to total genetic diversity

	Caballero and Toro (2002)			Petit *et al.* (1998)		
	Total (%)	Within Pop. (%)	Between Pop. (%)	Total (%)	Within Pop. (%)	Between Pop. (%)
KRK	–0.086	–0.079	–0.007	0.232	0.223	0.009
ELM	–0.026	–0.008	–0.019	–0.055	–0.246	0.190
KAS	–0.244	0.136	–0.379	–0.015	–0.313	0.298
DMR	–0.231	–0.275	0.044	0.140	0.223	–0.084
MNG	0.039	–0.022	0.061	0.256	0.223	0.033
GND	–0.107	–0.045	–0.062	0.096	–0.313	0.408
IBR	0.143	0.127	0.016	–0.199	–0.045	–0.154
AKS	–0.035	0.174	–0.210	0.482	–0.246	0.728
GZP	–0.060	–0.009	–0.051	0.370	0.491	–0.121

The results of the FCA analysis where the relation between populations is presented on a three dimensional plane are given in Fig. 1. According to FCA analysis, goat populations from eastern part of Antalya (GZP, IBR, AKS and GND districts) were closer than other populations. KRK, ELM, KAS and DMR populations in the west of Antalya clustered in a different region.

According to FCA analysis, Hair goat populations from eastern part of Antalya clustered closer than other populations. Other populations that were raised in the districts in the west of Antalya clustered more distant from each other in a different place. MNG population is located between these two groups. The results of FCA analysis are not surprising, when the geographic location of the districts in region and Hair goat production systems are considered. The districts of IBR, GND and AKS are very close to each other at altitude of approximately 900–1,000 m above sea level, located in the eastern part of Antalya in the inner part of the Taurus Mountains. These three districts are relatively

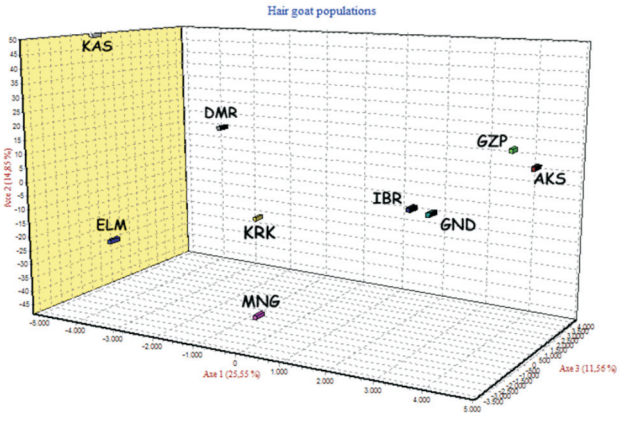

Fig. 1. Factorial correspondence Analysis based on reared populations in districts.

more closer and closed districts than other districts. Therefore, goat breeders in these three districts may exchange more breeding material compared to other districts. According to the results of FCA analysis, it is seen that there is a relationship between the populations raised in districts in the west of Antalya. In fact, this result is acceptable. It can be due to gene flow between populations because of breeding material exchange among farmers.

In conclusion, in this study using 20 microsatellite loci in Hair goat populations raised in 9 districts of Antalya, high genetic diversity and low level of inbreeding were determined. The results obtained from this study show that Hair goats reared in different districts of Antalya have great potential for breeding studies and for adaptation to the environmental conditions that will possibly change in the future. Especially, available genetic variation in KAS and AKS populations should be conserved.

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