View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Szeged

ANALYSIS OF GENETIC STRUCTURE OF SOME NATIVE TURKISH GOAT BREEDS BY 20 MİCROSATELLITE MARKERS

ÖZGECAN KORKMAZ AĞAOĞLU[1], OKAN ERTUĞRUL[2]

[1]Mehmet Akif Ersoy University, Faculty of Veterinary Medicine,
Department of Animal Science, Istiklal Location, 15030,
Burdur, Turkey
ozgecanagaoglu@mehmetakif.edu.tr
[2]Ankara University, Faculty of Veterinary Medicine,
Department of Genetics, Irfan Bastug Street, Diskapi, 06110
Ankara, Turkey
ertugrul@veterinary.ankara.edu.tr

ABSTRACT
Genetic diversity, genetic relationship and bottleneck were evaluated in Angora, Kilis, Honamli, Hair and Norduz goat breeds using 20 microsatellite markers. Analyses revealed that the average number of alleles per locus (15.65 allele/locus) and levels of heterozygosity (0.5192–0.9400) were fairly high. The calculated overall FIS value for all populations was 0.03656 ± 0.033 and it was not significant. All the populations were in the Hardy–Weinberg equilibrium. Gene exchange among populations was consistently high, its rate being Nm = 8.07 migrants per generation. According to FST values, a medium level of genetic diversity was found between the Angora goat breed and other breeds. Among the other breeds, genetic diversity was low and this diversity was statistically significant. Results of various analyses, such as allelic variation analysis, heterozygosity analysis, F statistics, STRUCTURE test and factorial correspondence analysis, indicated that the Angora goat breed is different than the other goat breeds. Furthermore, analysis showed that the other native goat breeds could not be distinguished from each other; these breeds were grouped together. The results obtained from the analysis of 20 microsatellite loci indicated that goat breeds other than the Angora goat breed cannot be genetically distinguished from each other.

Keywords: Genetic Diversity, Microsatellites, Native Turkish Goat Breeds, Population Structure, Bottleneck

INTRODUCTION

Goats (Capra hircus) are an important domestic animal because they were one of the first animal species to be domesticated (LUIKART et al., 2001; FERNANDEZ et al., 2006) and because of their ability to rapidly adapt to different environmental conditions. Goat breeding is one of the most important agricultural activity and source of livelihood in rural areas in Turkey (ERTUĞRUL et al., 1995). Native goat breeds in Turkey include the Angora, Kilis, Honamli, Hair and Norduz goat breeds (AKÇAPINAR, 1994). The Kilis, Honamli, Hair and Norduz goat breeds have some phenotypic similarities, but the Angora goat breed is different. Molecular genetics characterization with adequate number of microsatellite loci has not yet been done for these breeds. Hence, it is essential to genetically characterize and describe the genetic diversity of these native breeds. Many studies (DALVIT et al., 2008; CHAUDHARI et al., 2009) have been conducted to investigate the genetic diversity of farm animals, namely cattle and sheep, but studies on the genetic diversity of goat breeds are only recently being done in greater numbers. Some Turkish goat breeds have been used in different studies (LUIKART et al., 1999; CANON et al., 2006), but those studies either had a low number of samples or they had less than 20 microsatellite loci. Furthermore, new studies on genetic diversity that included these goat breeds have become more interesting to the scientific world because the earlier studies did not evaluate any breeds specific to Turkey, such as the Norduz goat, and because Anatolia is geographically close to major domestication centers. Turkey has rich genetic diversity because it is located between the

continents of Europe, Asia and Africa and functions as a bridge between them. Goat stock in Turkey numbered around 6,293,233 head (TUIK, 2011), which is almost 20% of small ruminants in Turkey. However, the number of goats has decreased dramatically since the 1990s (TUIK, 2011). The first step for the conservation and exploitation of domestic animal biodiversity is comprehensive knowledge of the existing genetic variability and how this variability is divided among breeds (IAMARTINO et al., 2005). For this reason, it is important and urgent to determine the genetic diversity of native Turkish goat breeds. The purpose of this study was to use 20 microsatellite markers to determine genetic diversity, genetic relationships and bottleneck in 5 native goat breeds raised in Turkey. The goal of this trial was to contribute to population genetics studies in Turkey using microsatellite markers and to make sure the method can be executed in the laboratory. The goal was also to achieve preliminary molecular identification using 20 microsatellite markers on the primary DNA gene bank, which was created by TURKHAYGEN-I project staff and which contains most of the native Turkish animal genetic resources.

MATERIAL AND METHOD

A total of 251 blood samples were collected from 5 different goat breeds in natural habitats. The sample size for each breed was: 50 Angora goats, 51 Kilis goats, 49 Honamli goats, 52 Hair goats and 49 Norduz goats. The goats were not blood related (according to animal pedigrees and breeders informations). Blood samples collected from the goat breeds were placed into an EDTA tube. Genomic DNA was extracted from 10 ml blood samples using the standard phenol chloroform method (SAMBROOK et al., 1989). Multiplex PCR methods were used (KORKMAZ AĞAOĞLU et al., 2010, 2011). Fragments were resolved on a Beckman Coulter CEQ-8000 Genetic Analyser. The following were calculated for each of the 20 microsatellite loci analyzed: the number of alleles (nA), frequencies of alleles and null alleles, average number of migrants per generation (Nm) (Nm≈(1- FST) ⁄ 4FST) (ALLENDORF AND LUIKART 2007), observed (Ho) and expected heterozygosity (unbiased – He, Hnb), Wright's F-statistics (WEIR AND COCKERHAM, 1984), polymorphic information content (PIC) (BOTSTEIN et al., 1980), Hardy–Weinberg equilibrium (HWE), genetic distances, phylogenetic tree (NEI et al., 1983), factorial correspondence analysis (LEBART et al., 1984), the STRUCTURE test, and the Bottleneck test. Genetix (v4.05) (BELKHIR et al., 2004), PowerStats V12 (BRENNER AND MORRIS, 1990), Genepop (RAYMOND AND ROUSSET 1995), STRUCTURE (PRITCHARD et al., 2000), Bottleneck v1.2.02 (CORNUET AND LUIKART, 1996) etc. programs were used for analysis.

RESULTS

In this study, a total of 313 alleles were observed. *Table 1* shows the observed number of alleles, observed and expected heterozygosities, PIC values as well as null allel frequencies for all the populations.

The average number of alleles per locus was 15.65. In native Turkish goat breeds, the number of observed alleles varied from 10.45 (Honamli goat breed) to 11.8 (Angora goat breed). The values were higher than observed in goat breeds from the Czech Republic (JANDUROVÁ et al., 2004) and in Egyptian and Italian goat breeds (AGHA et al., 2008). It is also higher than the values reported for other Indian, Chinese and Swiss goat breeds (FATIMA et al., 2008; QI et al., 2009; GLOWATZKI-MULLIS et al., 2008). The average observed heterozygosity between the populations was 0.78. This value in this study was

higher than that reported for the Kutchi breed of goat (0.59) (DIXIT et al., 2008), the Gohilwari breed of Indian goat (0.505) (KUMAR et al., 2009) and the Gujarat (India) goat breed (0.61) (FATIMA et al., 2008).

Table 1. Genetic variability parameters in native Turkish goat breeds.

Lokus	NA[a]	Hnb	Ho	PIC[b]	NAF[c]
BM1818	14	0.8526	0.8327	0.83	0,0235
CSRD247	16	0.8617	0.8487	0.84	0,0139
HSC	19	0.9046	0.8400	0.89	0,0335
ILSTS11	10	0.7589	0.7697	0.72	0,0175
ILSTS30	16	0.8513	0.7810	0.83	0,0342
INRA005	9	0.6351	0.6145	0.57	0,0254
INRA23	14	0.8754	0.8691	0.86	0,0083
MAF65	21	0.8445	0.8166	0.82	0,0225
MAF70	12	0.8318	0.8218	0.80	0,0198
OARAE54	16	0.8373	0.8244	0.81	0,0111
OARCP34	15	0.8540	0.8520	0.83	0,0060
OARFCB20	13	0.7598	0.6822	0.72	0,0423
OARFCB48	13	0.8358	0.8396	0.81	0,0071
OARFCB304	24	0.7750	0.7449	0.75	0,0133
SRCRSP1	19	0.7883	0.7331	0.75	0,0311
SRCRSP5	13	0.8538	0.8284	0.83	0,0355
SRCRSP8	16	0.7815	0.7290	0.75	0,0352
SRCRSP15	14	0.7280	0.7057	0.69	0,0211
SRCRSP23	19	0.8505	0.8238	0.83	0,0117
TGLA53	20	0.7979	0.7338	0.77	0,0327
Mean	15.65	0.8139	0.7846	0.78	0.0212

[a] Number of alleles, [b]Polymorphic information content, [c]Null allele frequency estimated

The statistical evaluation of informativeness of a marker is defined by PIC values, which varied between 0.57 (INRA005) and 0.89 (HSC) with a mean PIC of 0.78 across the populations. Genetic markers exhibiting PIC values higher than 0.5 are considered to be informative in genetic population analysis (BOTSTEIN et al., 1980). For this reason, genetic diversity studies may prefer these loci. The Wright's F-statistics for each breed, the genetic distance between populations and gene flow (Nm) in brackets were as shown in *Table 2.*

Table 2. Estimated pairwise FST and Nm between populations in brackets (above diagonal) and Nei's D_A genetic distance (below diagonal).

	Ankara	Kilis	Honamlı	Kıl	Norduz
Ankara	-	0.05734***(4.11)	0.05788*** (4.07)	0.05790*** (4.06)	0.06196*** (3.78)
Kilis	0.1520	-	0.01382*** (17.84)	0.01025*** (24.14)	0.01059*** (23.36)
Honamlı	0.1589	0.0813	-	0.00492*** (50.56)	0.01470** (16.76)
Kıl	0.1481	0.0643	0.0587	-	0.00587*** (42.34)
Norduz	0.1570	0.0712	0.0803	0.0592	-

*** ($P < 0.001$)

Gene exchange among populations was consistently high, its rate being Nm = 8.07 migrants per generation greater than the critical value of Nm = 1.0. The gene flow ranges from 3.78 to 50.56 between pairs of populations. The highest Nm value (50.56) was observed between Honamli and Hair breeds, indicating high rate of genetic flow between the populations. The lowest Nm values were estimated between Angora and Norduz breeds, indicating minimal genetic flow between Angora and Norduz. F_{IS} was calculated from the data values and the values were between 0.01621 and 0.04951. The calculated overall FIS value for all populations was 0.03656 ± 0.033 and it was not significant. All

the populations were in the Hardy–Weinberg equilibrium. According to Nei's D_A genetic distance values, the highest level of genetic distance was found between the Angora goat breed and other breeds. This result is compatible with the other test results. For STRUCTURE analysis, the most appropriate number of clusters for modeling the data was five. The axes in the FCA test also indicated that the Angora goat breed is grouped separately from the other breeds. The native Turkish goat breeds (except for Angora) are not completely separated from each other. The result of this analysis is similar to those obtained from other analyses *(Fig. 1).*

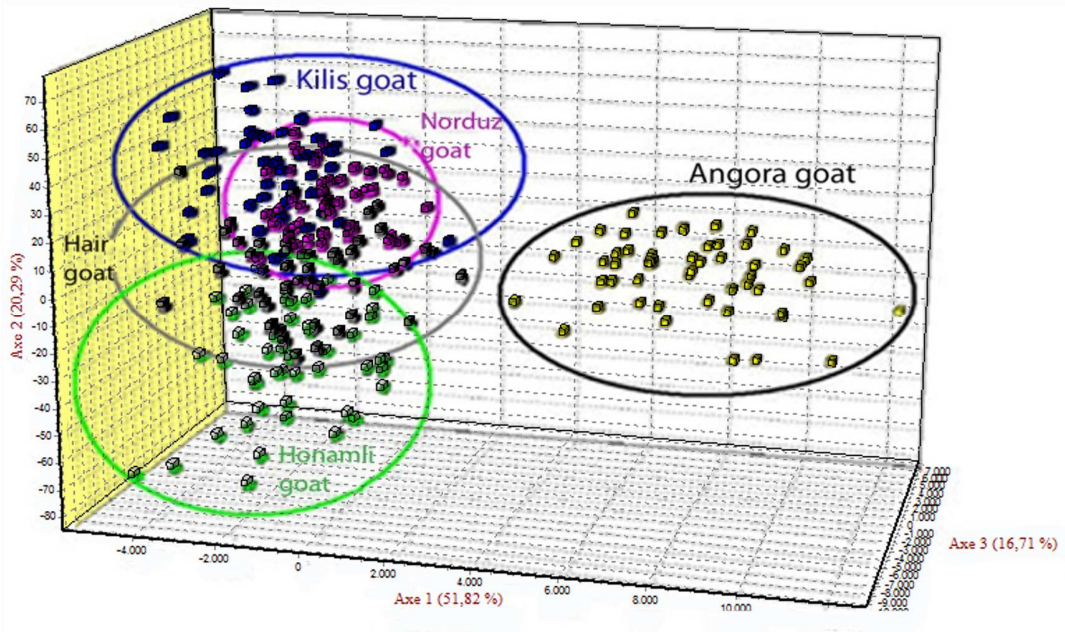

Figure 1. Graphic representation of the factorial correspondence analysis of five populations from Turkey.

The two phase mutation model under Wilcoxon's signed rank test and shift mode test were used to investigate any recent bottleneck (heterozygosity excess) in native Turkish goat populations. In a population at mutation-drift equilibrium, there is approximately an equal probability that a locus shows genetic diversity excess or deficit. GLOWATZKI-MULLIS et al. (2008) reported genetic bottleneck in the Valais Blackneck goat breed. Bottleneck has not been reported in Zalawadi and Gohilwadi goat populations, whereas mild bottleneck has been reported recently for the Surti breed by FATIMA et al. (2008). It is vital that native Turkish goat breeds have high genetic diversity. Unfortunately, the number of native goat breeds is continually decreasing due to numerous factors, including certain procedures performed by breeders in Turkey to increase efficiency (uncontrolled mating etc.), certain breeding programs that have been implemented, population growth, the diminished importance of certain yield factors (such as the yield of Angora goat mohair), and the fact that the value of goats has dropped because they are said to be harmful to forest vegetation. Native Turkish goat breeds have not undergone bottleneck according to the Wilcoxon sink-rank test in TPM and the mode shift test. However, numbers of native Turkish goats (especially the Angora, Honamli and Norduz goat breeds) have decreased significantly in recent years. In this regard, registered breeds should be kept pure, and breeders should be informed about this issue.

CONCLUSIONS

The data from this study showed that a considerable amount of information regarding genetic diversity and relationships in native Turkish goat breeds can be determined using microsatellite markers recommended by ISAG/FAO. Furthermore, the genetic material stored in the DNA bank made it possible to ascertain molecular characterization through the use of microsatellite markers. Moreover, this data provided important information for conservation programs and could be utilized to define breeding strategies.

ACKNOWLEDGEMENTS

This research was supported by the TUBITAK-KAMAG No. 106G005 (117) TURKHAYGEN-I (In Vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I) project. The authors would like to thank the staff of the TURKHAYGEN-I Project. In addition, this research is a summary of the PhD thesis by Özgecan Korkmaz Ağaoğlu.

REFERENCES

AGHA, S.H., PILLA, F., GALAL, S., SHAAT, I., D'ANDREA, M., REALE, S., ABDELSALAM, A.Z.A., LI, M.H., (2008): Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. J. Anim. Breed. Genet. 125, 194–200.

AKÇAPINAR, H., (1994): Keçi Yetiştiriciliği Ders Notu. A.Ü. Veteriner Fakültesi Zootekni Anabilim Dalı.

ALLENDORF F.W., LUIKART G., (2007): Conservation and the Genetics of Populations, 1st edn. MA, USA: Wiley-Blackwell.

BARKER, J.S.F., (1994): A global protocol for determining genetic distance among domestic livestock breeds. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, vol. 21, pp. 501–508.

BELKHIR, K., BORSA, P., CHIKHI, L., GOUDET, J., BONHOMME, F., (2004): GENETIX 4.05 WindowsTM Software for Sample Genetics. Laboratoire Genome, Populations, Interactions. University of Montpellier, France, Universite Montpellier II//www.univ-montp2.fr/~genetix/genetix/genetix.htm//December 2004.

BOTSTEIN, D., WHITE, R.L., SKOLNICK, M., DAVIS, R.W., (1980): Construction of a genetic linkage map in man using restriction fregment length poly-morphism. Am. J. Hum. Genet. 32, 324–331.

BRENNER, C., MORRIS, J., (1990): Paternity index calculations in single locus hypervariable DNA probes:validation and other studies. In: Proceedings for the International Symposium on Human Identification 1989, Promega Corporation, Madison, WI, pp. 21–53.

CANON, J., GARCIA, D., GARCIA-ATANCE, M.A., OBEXER-RUFF, G., LENSTRA, J.A., AJMONE-MARSAN, P., DUNNER, S., (2006): Geographical partitioning of goat diversity in Europe and the Middle East. Anim. Genet. 37 (4), 327–334.

CHAUDHARI, M.V., PARMAR, S.N.S., JOSHI, C.G., BHONG, C.D., FATIMA, S., THAKUR, M.S., THAKUR, S.S., (2009): Molecular characterization of Kenkatha and Gaolao (Bos indicus) cattle breeds using microsatellite markers. Anim. Biodivers. Conserv. 32 (2), 71–76.

CORNUET, J.M., LUIKART, G., (1996): Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

DALVIT, C., SACCA, E., CASSANDRO, M., GERVASO, M., PASTORE, E., PIASENTIER, E., (2008): Genetic diversity and variability in Alpine sheep breeds. Small Ruminant Res. 80, 45–51.
DIXIT, S.P., VERMA, N.K., AHLAWAT, S.P.S., AGGARWAL, R.A.K., KUMAR, S., CHAN-DER, R., SINGH, K.P., (2008): Molecular genetic characterization of Kutchi breed of goat. Curr. Sci. 95 (7), 946–952.
ERTUĞRUL, M., AKMAN, N., ELİÇİN, A., ARIK, Z., (1995): Küçükbaş¸ hayvansal ürünler tüketim projeksiyonları ve üretim hedefleri. In: TMMOB Ziraat Mühendisleri Odası, Türkiye Ziraat Mühendisliği IV, Teknik Kongresi, Ankara, pp. 753–770.
FATIMA, S., BHONG, C.D., RANK, D.N., JOSHI, C.G., (2008): Genetic variability and bottleneck studies in Zalawadi, Gohilwadi and Surti goat breeds of Gujarat (India) using microsatellites. Small Ruminant Res. 77, 58–64.
FERNANDEZ, H., HUGHES, S., VIGNE, J.D., HELMER, D., HODGINS, G., MIQUEL, C., HANNI, C., LUIKART, G., TABERLET, P., (2006): Divergent mtDNA lineages of goats in an Early Neolithic site, far from the initial domestication areas. PNAS 103, 15375–15379.
GLOWATZKI-MULLIS, M.L., MUNTWYLER, J., BAUMLE, E., GAILLARD, C., (2008): Genetic diversity measures of Swiss goat breeds as decision-making support for conservation policy. Small Ruminant Res. 74, 202–211.
IAMARTINO, D., BRUZZONE, A., LANZA, A., BLASI, M., PILLA, F., (2005): Genetic diversity of Southern Italian goat populations assessed by microsatellite markers. Small Ruminant Res. 57, 249–255.
JANDUROVÁ, O.M., KOTT, T., KOTTOVÁ, B., CZERNEKOVÁ, V., (2004): Seven microsatellite markers useful for determining genetic variability in White and Brown Short-Haired goat breeds. Small Ruminant Res. 52, 271–274.
KORKMAZ AĞAOĞLU, Ö., ÇINAR KUL, B., AKYÜZ, B., ÖZKAN, E., ERTUĞRUL, O., EROL, H., (2010): Keçi Türünde Mikrosatellit Polimorfizminin Belirlenmesinde Farklı Çoklu-PZR (Multipleks PCR) Sistemleri. Vet. Hekim. Der. Derg. 81 (2), 21–27.
KORKMAZ AĞAOĞLU, Ö., ÇINAR KUL, B., AKYÜZ, B., ÖZKAN, E., ERTUĞRUL, O., (2011): Different four PCR-multiplex systems via twenty microsatellite loci in goat. Curr. Opin. Biotechnol. 22 (Suppl. 1), S51.
KUMAR, S., DİXİT, S.P., VERMA, N.K., SİNGH, D.K., PANDE, A., KUMAR, S., CHANDER, R., SİNGH, L.B., (2009): Genetic diversity analysis of the Gohilwari breed of Indian goat (Capra hircus) using microsatellite markers. Am. J. Anim. Vet. Sci. 4 (3), 49–57.
LEBART, L., MORINEAU, A., WARWICK, K., (1984): Multivariant Descriptive Statistical Analysis. John Wiley and Sons, New York, USA.
LUIKART, G., BIJU-DUVAL, M.P., ERTUGRUL, O., ZAGDSUREN, Y., MAUDET, C., TABERLET, P., (1999): Power of 22 microsatellite markers in fluores-cent multiplexes for parentage analysis in goats (Capra hircus). Anim. Genet. 30 (6), 431–438.
LUIKART, G., GIELLY, L., EXCOFFIER, L., VIGNE, J.D., BOUVET, J., TABERLET, P., (2001): Multiple maternal origins and weak phylogeographic structure in domestic goats. Proc. Natl. Acad. Sci. U.S.A. 98, 5927–5932.
NEI, M., TAJIMA, F., TATENO, Y., (1983): Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19, 153–170.
PRITCHARD, J.K., STEPHENS, M., DONNELLY, P., (2000): Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
QI, Y., LUO, J., HAN, X.F., ZHU, Y.Z., CHEN, C., LIU, J.X., SHENG, H.J., (2009): Genetic diversity and relationships of 10 Chinese goat breeds in the Middle and Western China. Small Ruminant Res. 82, 88–93.

RAYMOND, M. AND ROUSSET, F. (1995): GENEPOP (version 1.2) population genetic software for exact tests and ecumenicism. J. Heredity, 86: 248-249. (website: http://www.cefe.cnrs-mop.fr/).
RYDER, M.L., (2001): Textile fibres from goats. In: Reeve, E.C.R. (Ed.), Encyclopedia of Genetics. Htzroy Dearborn Publishers, USA, pp. 379–381.
SAMBROOK, J., FRITSCH, E.F., MANIATIS, T., (1989): Molecular cloning: A laboratory manual, vol. 3., 2nd ed Cold Spring Harbor Laboratory, Cold-Spring Harbor, New York.
TUIK, (2011): Tarım Istatistikleri. http://www.tuik.gov.tr (accessed 20.10.2011.).
WEIR, B.S., COCKERHAM, C.C., (1984): Estimating F-statistics for the analysis of population structure. Evolution 38 (6), 1358–1370.
YALÇIN, B.C., (1990): Koyun Keçi Hastalıkları ve Yetiştiriciliği Kitabı. TÜM VET Hayvancılık Hizmetleri Yayınları 2 (456), Teknografik Matbaası, Istanbul.