The Journal of Animal & Plant Sciences, 21(3): 2011, Page:459-464
ISSN: 1018-7081

DETERMINATION OF GENETIC DISTANCE BETWEEN TURKISH SHEEP BREEDS WITH VARIOUS METHODS USING SOME BLOOD PROTEIN LOCI

M. Ozdemir, O. C. Bilgin, N. Esenbuga

Department of Animal Science, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey
Corresponding author e-mail: ozdemirm@atauni.edu.tr

ABSTRACT

This study was conducted to estimate genetic distances among Akkaraman, Morkaraman, Awassi, Merino, Karakul, Kivircik, Imroz, Daglic and Karayaka sheep breeds reared in Turkey based on simple polymorphic blood protein types by using various methods including horizontal strach gel electrophoresis and polyacrilamyde gel electrophoresis (PAGE). NJ and UPGMA trees were constructed based on genetic distances computed by Nei, Cavalli-Sforza and Reynolds methods. Phylogenetic trees constructed on the polymorphic systems of the populations using the methods of Nei and Reynolds were found similar whereas Cavalli-Sforza was different figure. All the trees produced by method of UPGMA exhibited two large clusters of sheep populations but NJ method yielded small groups of the breeds. As a result that we have seen on the phylogenetic tree diagrams the fat-tailed sheep were clearly separeted from the others.

Keywords: Blood polymorphism, Sheep, Genetic diversity, Phylogenetic tree.

INTRODUCTION

Livestock sector plays a significant role in Turkey and is essential for the nutrition of rural population. Sheep are the main livestock of nomadic people. The meat from sheep is an important source of daily food consumption accounting for approximately 20% of the total red meat production in Turkey. Typically small ruminant systems are comprised of small-sized flocks that are managed with family labor. Indigenous breeds are the main components of these systems. For centuries, Anatolian farmers have used the native breeds to produce milk, meat and wool (TurkStat 2008).

Approximately 87% of Turkey's sheep population (20.6 million heads) is fat-tailed breeds. Akkaraman and Morkaraman sheep are the most numerous and make up nearly 65% of the total sheep population. These sheep are thought to have evolved through natural selection under harsh environmental conditions. Akkaraman is a fat-tailed, indigenous breed constituting 40.8 % of the sheep population and reared extensively in middle Anatolia. Morkaraman is estimated at about 5.09 million heads. The sheep population of the eastern and northeastern parts of Turkey consists predominantly of the Mokaraman breed. Awassi is also a fat-tailed breed reared extensively in southern part of Turkey and it is estimated to be about one million Heads. One of the fat tailed breed, Daglic is grown on the mountainous terrain of the region Ege and Marmara and is estimated to be about 3.9 million heads. Karakul was brought into Turkey in 1929 from Russia. Karakul is fat tailed breed (TurkStat 2008).

Thin-tailed Kivircik is the main native sheep breed of Thrace and Marmara regions of Turkey and there are about 1.7 million heads. Karayaka having long thin tail is raised on coastline of Black Sea region with a total population of approximately one million sheep. Imroz also have long thin tail and are raised in Gokceada and Canakkale regions. Imroz is estimated at about 70.000 heads. The German Mutton Merino was brought into Turkey in the 1930 to increase live weight and fleece quality of indigenous sheep breeds (TurkStat 2008).

Changes in morphological and physiological characteristics of animals are based on the variations in respective traits. The extent of genetic diversity among animals increases due to diverse environmental differences which give rise to random effects (Soysal *et al.* 2005). This variation diminishes in the course of selection for economically important traits suitable for market purposes and preventing others less important or undesirable ones using breeding methods. Subsequently, wild type genotypes have been transformed into highly productive domestic breeds of livestock by breeders through the application of different mating systems. Sheep was reportedly the first domesticated species in the region of the Zagros Mountains on the border of Turkey and Iran approximately 10.000 years ago (Legge 1996; Uerpmann 1996) from where it was distributed in different parts of the world (Tsunoda *et al.* 2006).

Contrary to early phylogenetic studies on domestic breeds emphasis on studying phenotypic variation was shifted to determining blood type, milk proteins, and other biochemical variations which is further advanced to molecular phylogenetics due to more precise nature of these methodologies (Ceriotti *et al.* 2003).

Blood proteins are reportedly associated with quantitative and adaptability traits and can be detected at or very shortly before birth (Dally *et al.* 1980). They are inherited as co-dominant autosomal traits in accordance with simple Mendelian principles (Dogru *et al.* 2008). Such polymorphic markers can be used as tools in animal husbandry for herd/flock registry, accurate paternal records, determine evolutionary relationship among different breeds, probing genealogical records, profiling breed origins, working out genetic distances, documenting variations in phenotypic frequencies and to make strategic decisions regarding future breeding goals (Dally *et al.* 1980; Dayıoglu and Dogrul 1989; Dellal 2001; Mert *et al.* 2003).

Hemoglobin and transferin loci under study are unambiguously named with known functions and products. The utilization polymorphic data on these traits by employing population genetics methods is substantiated by the fact that they are inherited as Mendelian dominant characters and attempts have been made to associate them with quantitative traits.

Polymorphic studies on haemoglobin (Hb) and transferrin (Tf) proteins in Turkish sheep have been reported by Dogrul 1985; Soysal *et al.* 1986; Dayıoglu and Dogrul 1989; Esenbuga *et al.* 1998; Dellal, 2001; Mert *et al.* 2003, Bulut 2004, Koban 2004 and Soysal *et al*., 2005. On the basis of genotypic information on protein level, several genetic studies on the phylogenetic relationship in protein genotyping of sheep and cattle breeds have been reported esleswhere (Dally *et al.* 1980; Ibegha-Awemu and Erhardt 2004; Tsunado *et al.* 2006, Rincon and Medrano 2003; Ibegha-Awemu *et al.* 2004; Rincon *et al.*, 2006; Dogru *et al.* 2008) .This study investigates genetic diversity between some improved and native sheep breeds raised in Turkey utilizing the genetic variation based on allele frequency data of transferrin (Tf) and haemoglobin (Hb) loci.

Further studies on genetic diversity studies in Turkish sheep breeds could therefore bring up some valuable data to confirm their genetic proximity to acclaimed Turkish ancestral populations. Most of these breeds are presently subjected to genetic conservation studies in Research Institutes of Turkey at present.

MATERIALS AND METHODS

Gene frequency data from retrospective studies (Dogrul 1985; Dayıoglu and Dogrul 1989; Esenbuga *et al.* 1998; Dellal 2001) was utilized to workout genetic distances among Akkaraman, Morkaraman, Awassi, Merino, Kivircik, Karakul, Daglic, Karayaka and Imroz sheep breeds raised in Turkey. The data on gene frequencies imported from cited studies is presented in table 1.

Table 1. Alleles frequencies of Transferrin and Haemoglobin variants of Turkish sheep breeds

System	Alleles	Akkaraman	Morkaraman	Awassi	Merino	Karakul	Kivircik	Imroz	Daglic	Karayaka
		(N)=(342)	(369)	(393)	(174)	(185)	(159)	(155)	(148)	(148)
	A	0.168	0.381	0.310	0.167	0.043	0.252	0.039	0.014	0.081
	B	0.405	0.196	0.340	0.179	0.152	0.236	0.345	0.223	0.267
Tf	M	0.235	0.195	0.172	0.273	0.273	0.104	0.174	0.230	0.115
	D	0.187	0.219	0.134	0.345	0.529	0.336	0.439	0.524	0.432
	E	0.004	0.011	0.044	0.035	0.002	0.072	0.000	0.010	0.105
	S	0.000	0.000	0.004	0.000	0.000	0.000	0.003	0.000	0.000
		(N)=(135)	(369)	(394)	(62)	(139)	(159)	(156)	(149)	(150)
Hb	A	0.000	0.089	0.096	0.081	0.000	0.075	0.352	0.114	0.140
	B	1.000	0.911	0.904	0.919	1.000	0.925	0.648	0.886	0.860

Based on allele frequency data presented in the above table, Nei, Cavalli-Sforza and Reynolds algorithms were used to determine genetic diversity among different sheep breeds of Turkey in addition to constructing matrix of genetic distance and Dendograms drawn based on genetic diversity matrix using Neighbor-joining (NJ; Saitou and Nei 1987) and the unweighted pair-group methods with arithmetic mean method (UPGMA; Sokal and Michener 1958). These procedures, including estimation of the genetic distance, were performed by using the Gendist and Neighbor software of PHYLIP (Felsenstein 1996).

RESULTS AND DISCUSSION

Based on the results presented in Table 1 allele frequencies of Tf and Hb systems in this study six Tf locus alleles were identified in all breeds. Most frequent of them being allele A in Morkaraman, allele B in Akkaraman and Awassi, and allel D in other breeds. Allele E was observed in all breeds except for Imroz while allele S in Imroz and Awassi only. Allele B was found to be most frequent in respect of Hb locus in all breeds investigated. Allele A was not found in Akkaraman and Karakul.

The genetic distance matrices of populations obtained by Nei, Cavalli-Sforza and Reynolds methods are presented in Table 2.

Table 2. Genetic distance matrices calculated by Nei, Cavalli-Sforza and Reynolds methods .

Method	Breeds	Akkaraman	Morkaraman	Awassi	Merino	Karakul	Kivircik	Imroz	Daglic
Nei	Morkaraman	0.0366							
	Awassi	0.0129	0.0138						
	Merino	0.0313	0.0275	0.0412					
	Karakul	0.0766	0.0852	0.1090	0.0184				
	Kivircik	0.0319	0.0176	0.0244	0.0163	0.0461			
	Imroz	0.0337	0.0746	0.0667	0.0244	0.0204	0.0300		
	Daglic	0.0656	0.0899	0.1021	0.0219	**0.0028**	0.0423	0.0095	
	Karayaka	0.0442	0.0627	0.0622	0.0208	0.0220	0.0160	0.0084	0.0135
Cavalli-Sforza	Morkaraman	0.0281							
	Awassi	0.02001	0.0158						
	Merino	0.0288	0.0235	0.0379					
	Karakul	0.0635	0.0824	0.1113	0.0272				
	Kivircik	0.0407	0.0222	0.0247	0.0201	0.0696			
	Imroz	0.0375	0.0835	0.0880	0.0423	0.0200	0.0656		
	Daglic	0.0661	0.1036	0.1173	0.0383	**0.0065**	0.0734	0.0141	
	Karayaka	0.0569	0.0695	0.0620	0.0275	0.0465	0.0192	0.0432	0.0343
Reynolds	Morkaraman	0.0596							
	Awassi	0.0219	0.0229						
	Merino	0.0509	0. 0444	0.0635					
	Karakul	0.1289	0.1391	0.1673	0.0360				
	Kivircik	0.0514	0.0288	0.0388	0.0263	0.0813			
	Imroz	0.0596	0.1190	0.1068	0.0434	0.0412	0.0522		
	Daglic	0.1129	0.1451	0.1585	0.0419	**0.0062**	0.0753	0.0198	
	Karayaka	0.0722	0.0974	0.0953	0.0348	0.0429	0.0270	0.0161	0.0271

The genetic distance estimates between different breeds were changed as 0.1090 from 0.0028, to 0.1673 from 0.0062, and to 0.1173 from 0.0065 due to Nei, Cavalli-Sforza and Reynolds methods respectively. Awassi and Daglic breeds appeared to be genetically far distant from each other with respect to Cavalli-Sforza while Awassi and Karakul with respect to Nei and Reynolds (Table 2), whereas Daglic and Karakul revealed to be genetically most closer with all methods.

These findings on genetic variability of Turkish breeds were as extensive as reported in different European breeds (Koban 2004, Diez-Tascon *et al.* 2000) reporting genetic distance estimate of 0.209 between the Spanish and German Merino. Similarly intimate genetic distance of 0.139 was reported between the French Mutton and German Mutton Merino (Arranz *et al.* 1998). Estimated the genetic distance among 20 variants, and their UPGMA dendogram of relationships indicated a clear separation between the African and German breeds and a closer relationship between breeds in each group by Ibega-Awemu and Erhardt (2004) which was indicative of a relatively large divergence between European and African sheep breeds. In a similar study using data on five protein-coding loci, Mwacharo *et al.* (2002) obtained closer estimates of genetic distance between Kenyan sheep breeds (0.044–0.169) compared to Kenyan breeds and the Merino (0.044–0.283) as demonstrated by dendogram of relationships.

Most probable reason for an extensive genetic variability inference between Turkish breeds obtained in this study being comparable to different European breeds may be the recent decline of the sheep population sizes due to immigrate of localized family to the big city, decline of grassland and economic reasons, whereas closer genetic variability than expected might have arisen from the fact that sheep were probably first domesticated in southeastern Turkey approximately ten thousand years ago therefore warranting the need for further studies on Turkish sheep breeds in the nearest of ancestral populations which would substantiate to evolve conservation strategies for imminent implementation.

Phylogenetic trees based on allele frequencies of Tf and Hb loci were also constructed in this study mainly by NJ and UPGMA (Figure 1 and 2). Similar trees were produced by Nei and Reynolds with both Nj and UPGMA whereas the tree by Cavalli-Sforza was different from Nei and Reynolds and similar in NJ and PGMA. Although there was not a clear difference, Nei and Reynolds with NJ produced seven groups: (1) Akkaraman, (2) Morkaraman and Awassi, (3) Kivircik,

(4) Merino, (5) Karayaka, (6) Imroz and (7), Daglic and Karakul. On the other hand, Cavalli-Sforza changed the number of groups up to 8 placing Morkaraman as a separate group. Both Nei and Raynolds with UPGMA were revealed two large groups: (1) Akkaraman, Awassi, Morkaraman, Merino and Kıvırcık, and (2) Karakul, Daglic, Imroz and Karayaka but Cavalli-Sforza algorithm placed Karayaka to group 1 from group 2.

As an additional result of this study, it is virtually seen on all of the phylogenetic tree diagrams that the fat-tailed sheep were clearly separeted from the others. Fat-tailed sheep breeds (Akkaraman, Morkaraman, Awassi) were closer each other among all the breeds. Fat-tailed with a thin end sheep breeds (Karakul, Dagliç) were closer. Two of thin-tailed sheep breeds (Kıvırcık, Merino) were closer while the others (Imroz, Karayaka) were closer each other.

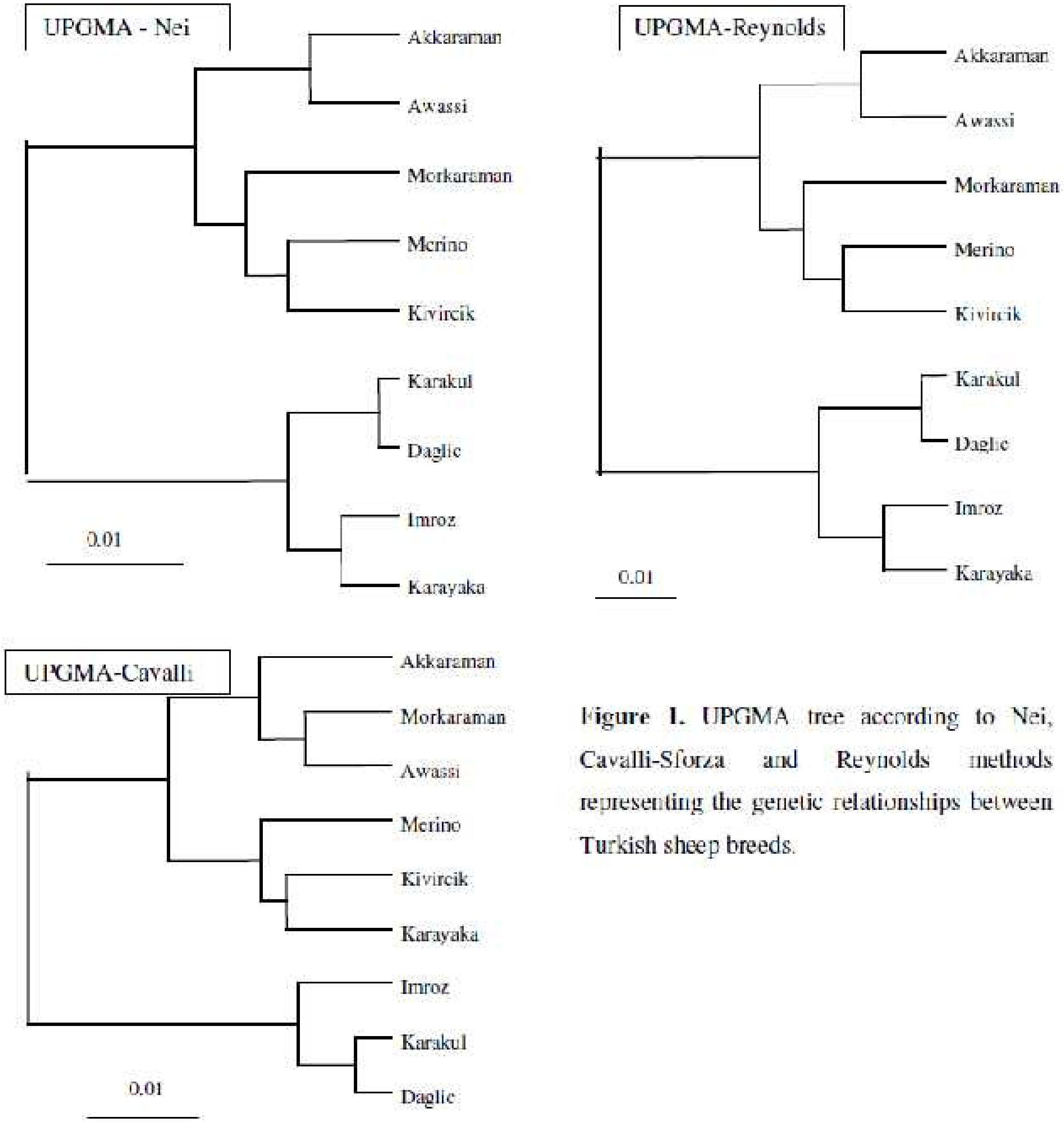

Figure 1. UPGMA tree according to Nei, Cavalli-Sforza and Reynolds methods representing the genetic relationships between Turkish sheep breeds.

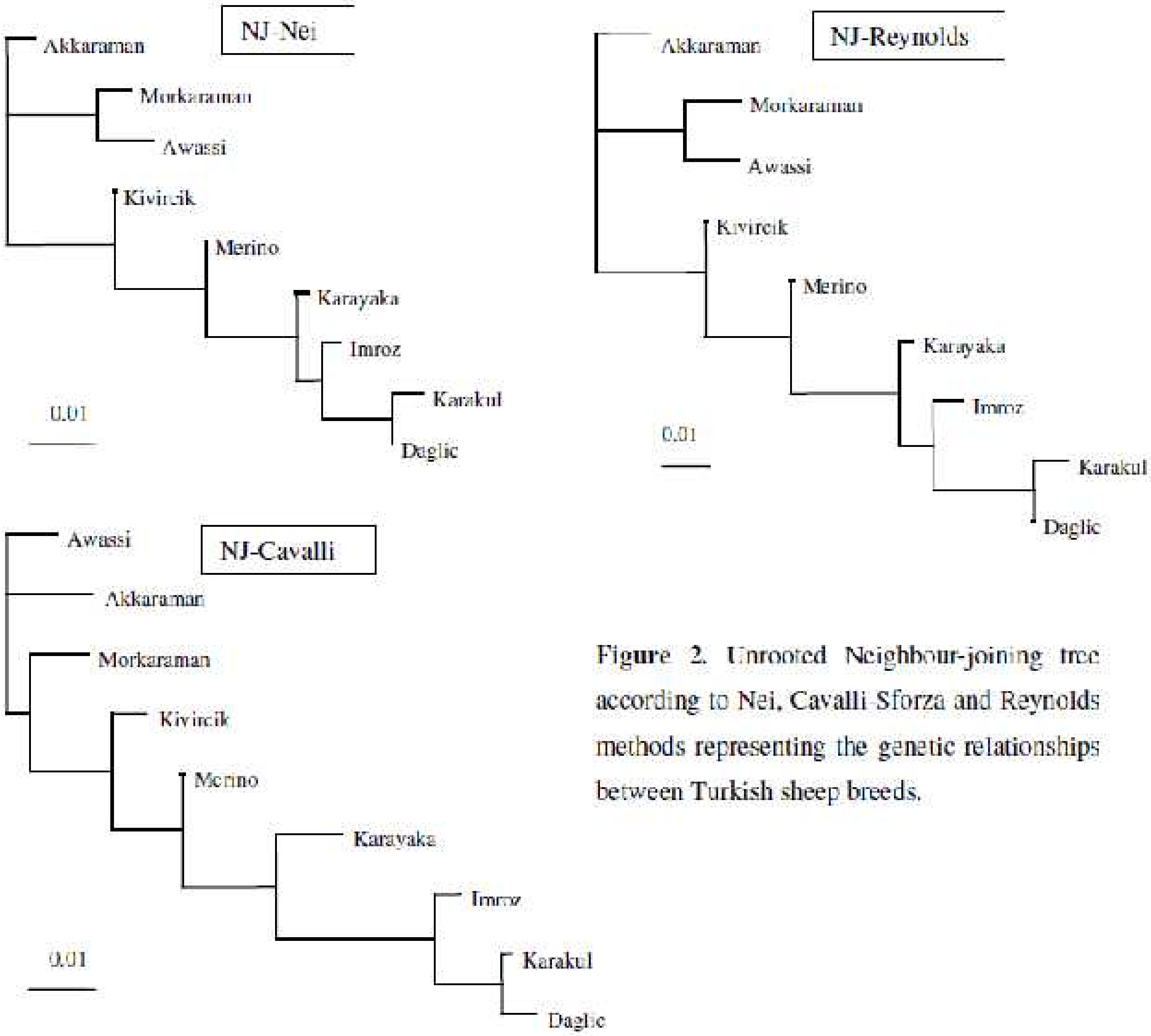

Figure 2. Unrooted Neighbour-joining tree according to Nei, Cavalli Sforza and Reynolds methods representing the genetic relationships between Turkish sheep breeds.

Conclusion: UPGMA and NJ methods were not similar in the results for cluster analysis and differed especially at the subgroup level as revealed in the present study which might be indicative that UPGMA method being more accurate because of forming two large divergent groups with three methods of calculating genetic distance compared to NJ method. In addition, the bootstrap values for the main nodes of the dendograms were computed for reference. The computed values were generally low.

Determination of genetic diversity among different breeds is imperative to develop conservation strategies of AnGR despite the fact that all breeds have arisen from the same origin, but the data on breed-to-breed and even a population-to-population variation for important genes and their frequencies can help to understand dynamics of genetic change owing to factors such as natural selection, breeding strategies and genotype x environment interaction. Considering this fact, it may be possible to distinguish between the breeds correctly by means of genetic frequencies of the polymorphic structures investigated. As a matter of fact a separation of fat-tailed sheep breeds from the others clearly appeared on all of the phylogenetic tree diagrams.

REFERENCES

Arranz J. J., Y. Bayon, and F. S. Primitivo (1998). Genetic relationships among Spanish breeds using microsatellites. Animal Genetics 29, 435-440.

Buchanan F. C., L. J. Adams, J. F. Littlejohn, J. F. Maddox, and A. M. Crawford (1994). Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics 22, 297-403.

Bulut Z. (2004). Investigation of genetic structure of some sheep breeds in Turkey by using microsatellites. Phd Thesis. Graduate School of Healty Science, Selcuk University. Konya.

Ceriotti G, A. Caroli, R. J. Rizzi, and C. Crimella (2003). Genetic relationships among taurine (Bos taurus) and zebu (Bos indicus) populations as revealed by blood groups and blood proteins. J. Anim. Breed. Genet. 120:57-67.

Dally M. R., W. Hohenboken, D. L. Thomas, and A. M. Craig (1980). Crossbred ewes lamb, wool and milk production and health-related traits in relationship between hemoglobin type and reproduction, J. Anim. Sci. 50: 418-427.

Dayıoglu H. and F. Dogrul (1989). Ataturk Universitesi koyun surulerinin transferin polymorfizmi yonunden genetik yapısı. J. Etlik Vet. Micr. 6: 25-33.

Dellal G. (2001). Effects of environmental and hereditary factors on lamb production traits in White Karaman and Anatolian Merino ewes. Turk. J. Vet. Anim. Sci. 26: 581-586.

Diez-Tascon C, R. P. Littlejohn, P. A. R. Almeida, and A. M. Crawford (2000). Genetic variation within the Merino sheep breed: Analysis of closely related populations using microsatellites. Anim. Genetics 31:243-251.

Dogru U., M. Ozdemir, M. I. Soysal, and M. Topal (2008). Determination of genetic distance between East Anatolian Red, Brown Swiss, Holstein and Simmental breeds of cattle using polymorphic systems. J. App. Anim. Res. 33: 145-148.

Dogrul F. (1985). Cesitli koyun ırklarında transferin ve hemoglobin tiplerinin dagilimi uzerine arastirma. J. Etlik Vet. Micr. Inst. 5:61-75.

Esenbuga N, H. Dayıoglu, and E. Emsen (1998). Ivesi ve Morkaraman surulerinde polimorfik kan ve sut protein sistemleri uzerine genetik ve sistematik arastirmalar. II. Ulusal Zootekni Kongresi. 22-25 Eylul, Bursa.

Felsentein J. (1993). PHYLIP. Phylogeny inference package. Version 3.5c. Department of genetics SK-50, Universty of Washington, Seattle, WA.

Ibegha.Awemu E. M. and G. Erhardt (2004). Genetic variations between African and German sheep breeds, and description of a new variant of vitamin D-binding protein. Small Rumin. Res. 55, 33-43.

Ibeagha-Avemu E. M., O. C. Jann, C. Weiman, and G. Erhardt (2004). Genetic diversity, introgression and relationships among west/central African cattle breeds. Genet. Sel. Evol. 36:673-690.

Koban E. (2004). Genetic diversity of native and Hybrid sheep breeds in Anatolia. PhD. Thesis. Graduate School of Natural and Applied Science, METU, Ankara.

Legge T. (1996). The beginnings of caprine domestication in Southwest Asia. In The Origins and Spread of Agriculture and Pastoralism in Eurasia, edited by D. Harris, pp. 238-262. UCL Press, London.

Mert N, H. Gunduz, V. Akgunduz, and M. Akgunduz (2003). Correlation between biochemical parameters and production traits in merino cross sheep. II. Hemoglobin and transferrin types. Turk J. Vet. Anim. Sci. 27:575-581

Mwacharo J. M., C. J. Otieno, and A. M. Okeyo (2002). Genetic variations between indigenous fat-tailed sheep populations in Kenya. Small Rumin. Res. 44, 173–178.

Rincon G. and J. F. Medrano (2003). Single nucleotide polymorphism genotyping of bovine milk protein genes using the tetra-primer ARMS-PCR. J. Anim. Breed. Genet. 120: 331-337.

Rincon G., E. Armstrong, and A. Postiglioni (2006). Analysis of the population structure of Uruguayan Creole cattle as inferred from milk major gene polymorphisms. Genet. Mol. Biol. 29(3):491-495.

Saitou N, and M. Nei (1987). The neighbor–joining method: A new mehod for reconstructing phylogenetic tree. Mol. Biol.Evol. 4, 406-425.

Sokal R. R. and C. D. Michener (1958). A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 28:1409.

Soysal M. I., E. Koban, E. Ozkan, V. Altunok, Z. Bulut, M. Nizamlioglu, and I. Togan (2005). Evolutinary relationship among three native and two crossbreed sheep breeds of Turkey: preliminary results. Revue Med. Vet. 156(5): 289-293.

Soysal M.I., K. Tanaka, and S. Suzuki (1986). Haemoglobin and transferin polymorphisms in some Turkish native sheep and their several crosses. Proceed. Japan Acad. 62(B1):38-40.

Uerpmann H. P. (1996). Animal domestication-accident or intention? In The Origins and Spread of Agriculture and Pastoralism in Eurasia, edited by D. Harris, pp. 227-237. UCL Press, London

TurkStat (Turkish Statistical Institute) (2008). Livestock Statistics, Url:http://www. turkstat.gov.tr

Tsunoda K, C. Hong, S. Wei, M. A. Hasnath, M. M. Nyunt, H. B. Rajbhandary, T. Dorji, H. Tumennasan, and K. Sato (2006). Phylogenetic relationships among indigenous sheep populations in East Asia based on five informative blood protein and nonprotein polymorphisms. Biochem.Genet. 44(7/8):287-306.