Evolutionary relationship among three native and two crossbreed sheep breeds of Turkey : preliminary results

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SUMMARY

The Turkish native sheep breeds, possibly being the neighbours of the earliest domesticated sheep populations, might be harbouring important genetic characteristics to be employed in the future for the improvement of sheep breeds. In order to design a conservation strategy, their genetic diversities must be determined. In the present study, based on three microsatellite loci, the genetic diversity of the Kivircik, Awassi, Akkaraman breeds (native) of Turkey as well as two of their crossbreeds Türkgeldi and Konya Merino were studied comparatively. It was observed that their heterozygoties are all high (0.6673-0.7822) compared to previously studied breeds, as expected for populations close to the center of domestication. Neighbour Joining (NJ) tree based on allele sharing distances indicated that the inertia of the breeds are not high. Yet, the genetic differentiations between the breeds based on pairwise F_{ST} (inbreeding coefficient) values are all significant. Furthermore, the three microsatellite loci could distinguish three groups of native breeds and their crossbreeds; 1) Awassi, 2) Kivircik-Türkgeldi and 3) Akkaraman-Konya Merino.

Keywords : Ovis - Turkish sheep breeds - microsatellite - genetic diversity.

RÉSUMÉ

Relation génétique entre trois races ovines natives de Turquie et trois races issues de croisements : résultats préliminaires. Par M.I. SOYSAL, E. KOBAN, ÖZKAN, V. ALTUNOK, Z. BULUT, M. NIZAMLIOGLU et I. TOGAN.

Les races de moutons originaires de Turquie, étant probablement les plus proches des premières populations de moutons domestiqués, pourraient présenter des caractéristiques génétiques importantes pour l'amélioration des races actuelles. Afin de mettre sur pied une stratégie de conservation, il est nécessaire de déterminer la diversité génétique de ces races anciennes. Dans notre étude, basée sur l'analyse de trois loci microsatellites, la diversité génétique de trois races de moutons originaires de Turquie : Kivircik, Awassi et Akkaraman ainsi que de deux races hybrides : Türkgeldi et Konya Merino ont été étudiées et comparées. Il apparaît que leurs niveaux d'hétérozygotie sont tous élevés (0.6673-0.7822) par rapport à ceux des races de moutons étudiées auparavant, comme attendu pour des populations proches du centre de domestication. Des arbres « Neighbour Joining » basés sur la distance des allèles partagés indique que l'inertie de ces races n'est pas élevée. Cependant, les différences génétiques entre ces races sont toutes significatives sur la base de leurs valeurs pairées de F_{ST} (coefficient de consanguinité). De plus, les trois loci microsatellites étudiés permettent de distinguer trois groupes comportant des races locales et des races hybrides : 1) Awassi, 2) Kivircik-Türkgeldi 3) Akkaraman-Konya Merino.

Mots-clés : Ovins - races ovines turques - microsatellite - diversité génétique.

Introduction

Archaeological [15, 18] and preliminary genetic studies [5, 10] indicated that sheep were probably first domesticated in Southeast Turkey approximately 10,000 years ago. Hence, the present day native Turkish sheep breeds may be the direct descendent or at least the nearest of ancestral populations.

There are more than 1400 modern domestic sheep breeds [24]. However, the Food and Agriculture Organisation of the United Nations (FAO) estimates that at least one breed of traditional livestock becomes extinct every week and more than 30% of European livestock are currently estimated to be endangered [12]. One of the major causes of extinction is the preference of one particular breed at one time and its spread at the expense of others as it was the case for the Holstein Friesian cattle [1]. Breeds are also lost by an indiscriminate cross breeding. With every extinction event, it is highly likely that unique characteristics (such as adaptation to local environmental conditions) are lost forever. Native Turkish breeds, perhaps genetically being closest to ancestral domestic populations, might be harbouring many more unique characteristics such as resistance to diseases and harsh environmental conditions, which can be employed in the future to improve the sheep breeds. Therefore, Turkish breeds may have higher priority in conservation than many other sheep breeds.

To develop conservation strategies for the breeds, their genetic diversity and genetic distinctness must be determined [4, 21]. For this purpose, different molecular techniques are used [4, 14, 19]. It has been established that microsatellite DNA markers are suitable markers for the description of breeds [20] and their highly polymorphic nature [13] have shown that there is significant differentiation between breeds of sheep [9, 10].

Microsatellite markers can also be used to identify genes associated with important economic traits such as milk yield and composition [11]. Furthermore, they are efficient for

establishing pedigrees in many cases [16, 25].

The aim of this study was to measure the genetic diversities that are present within three native and two crossbreed sheep breeds in Turkey using three highly polymorphic microsatellites. This study is the first one where microsatellite markers are used to estimate genetic diversity in Turkish domestic sheep breeds.

Materials and methods

BREEDS

Blood samples were obtained from 35 sheep of the native Kivircik breed of Turkey, 34 of the Türkgeldi breed (a crossbreed of Kivircik and East Frisian), 35 of the native Akkaraman breed of Central Anatolia, 35 of the Konya Merino breed (a crossbreed of Akkaraman and German Meat Merino) and 35 of the native Awassi breed. Kivircik breed sheep samples were collected from the Tekirdag-Inanli Agricultural Enterprise in Thrace. Türkgeldi breed sheep samples were obtained from the Sheep Research Unit of Thrace University, Agriculture Faculty and Department of Animal Science. The Akkaraman and Konya Merino sheep samples were taken from the Konya stud of Selçuk University in Central Anatolia. The Awassi samples were collected from the Gözlü Agricultural Enterprise in Konya, Central Anatolia.

MOLECULAR ANALYSIS

Blood samples were collected in 10 mL tubes containing K_2EDTA and stored at -20°C until the DNA was extracted by using the standard Phenol-Chloroform technique [23]. The microsatellite loci used in the study and their characteristics are given in Table I.

The PCR analyses were carried out using a Stratagene thermal cycler. The reaction mixture was composed of genomic DNA (100ng), 200 µm dNTPs, 2.0mM $MgCl_2$, 1X PCR buffer, 3 pmol forward and reversed primers and Taq DNA Polymerase (0.5 u/sample) in a total volume of 15.0 µL. The PCR reactions were carried out in 0.2 mL PCR plates with the following PCR conditions : 1 cycle of initial denaturation for 5 minutes at 94°C, 30 cycles of 45 seconds at 94°C, 45 seconds at annealing temperature, 1 minute at 72°C, and 1 cycle of final extension for 10 minutes at 72°C. In order to minimise the artefacts caused during amplification, one or more positive controls were used in each PCR reaction together with a negative control.

The amplification products were run on 6% denaturing polyacrylamide gel together with 50 bp and pBR322 *HaeIII* digest DNA size standards (MBI Fermentas). Then the amplified DNA fragments were visualised by silver staining. The sizes of each allele were estimated in accordance with the DNA size standards and standards obtained from the University of Cardiff.

DATA ANALYSIS

For each breed and for each locus, the number of alleles (n_A), the observed heterozygosity (H_o) and the unbiased expected heterozygosity (H_e) were calculated. For each breed, the average values of n_A, H_o, H_e, based on three loci were also computed. Finally, for each breed, F_{IS} (the inbreeding coefficient) of Wright's F statistics based on three loci were estimated and used to test the deviation from the Hardy-Weinberg equilibrium. The null distribution of F_{IS} was approximated by permuting alleles 1000 times within each breed. The significance level of F_{IS} was determined by comparing the observed value of F_{IS} with the distribution constructed by the permutations. Pairwise genetic differences between the breeds were measured by F_{ST} of Wright's F statistics using Weir and Cockerham's [26] approach. Significance of the differences was again tested by the permutation approach. All of the above computations were performed by GENETIX 4.0 [2].

To visualise the genetic relationships among the breeds Nei's standard genetic distance (D_S) [22] between the breeds were calculated, Neighbour Joining (NJ) tree was constructed and 1000 bootstrap re-sampling over loci were performed.

To visualise the distribution of the individuals within the breeds and hence to assess the distinctness of the breeds based on the three microsatellites studied, allele sharing distances [3] between all of the individuals were computed, NJ tree was constructed and 1000 bootstrap re-sampling over loci were performed.

Locus Name	Primer Sequence (5'-3')	PIC	Annealing Temperature°C	Chromosome Number	Genebank Accession Number	Reference
OarFCB20	Forward:GGAAAACCCCCATATATA CCTATAC Reverse:AAATGTGTTTAAGATTCCATACATGTG	0.80	63°C	2	L20004	[8]
OarFCB304	Forward:CCCTAGGAGCTTTCAATAAAGAATCGG Reverse:CGCTGCTGTCAACTGGGTCAGGG	0.54	63°C	19	L01535	[7]
MAF65	Forward:AAAGGCCAGAGTATGCAATTAGGAG Reverse :CCACTCCTCCTGAGAATAATATAACAG	0.62	60°C	15	M67437	[6]

TABLE I. — Characteristics of microsatellite loci used in the study : name of the loci, primer sequences, polymorphism information contents (PIC), annealing temperature, the chromosome numbers they belong to, genebank accession numbers and reference articles.

The distances (D_S and allele sharing distances) were calculated and NJ trees were constructed by *Populations* (v 1.0) software [17].

Results

Altogether 45 alleles were observed based on three microsatellites in five breeds and 174 individuals. Distributions of these alleles over the loci were as follows: 21 in OarFCB304, 14 in OarFCB20 and 10 in MAF65. Table II summarises the genetic diversity observed among the breeds. Single locus H_e values varied between 0.615 (MAF65, Türkgeldi) and 0.896 (OarFCB20, Kivircik). Average H_e values were very similar. The range was 0.7258 (Türkgeldi) - 0.7822 (Kivircik). However, the two lowest average H_e values were observed for Türkgeldi and Konya Merino. Similarly the average numbers of alleles per breed were very similar and they varied between 6.67 (Türkgeldi) and 9 (Akkaraman). Number of private alleles (i.e., the alleles observed only in one of the breeds) was zero in Konya Merino and 6 in Kivircik.

F_{IS} values of the breeds averaged over three loci are shown in Table III. When F_{IS} values were tested by permutation test none of them were found to be significant. Hence, breeds based on the samples seemed to be in Hardy-Weinberg equilibrium. In other words, there was not a significant deviation between the expected heterozygosity values, which were based on the absence of evolutionary forces; presence of large population, random mating, and the observed heterozygosity values.

Table IV presents the pairwise F_{ST} (measures the degree of genetic differentiation between the breeds) values of the breeds in the upper half triangle. The significance test (by permuting the data 1000 times) revealed that they are all significantly ($p<0.001$) different from each other. The lower triangle of Table IV exhibits the Nei's standard genetic distances between the breeds.

In order to visualise the genetic relatedness of the breeds Nei's D_S was used to construct the NJ tree, which is presented in Figure 1.

From the figure, it can be seen that three groups are formed within the breeds. Awassi forms the first group. The second group is formed by both Akkaraman and Konya Merino, which is the crossbreed obtained by crossing Akkaraman and German Meat Merino. The last group is composed of Kivircik and Türkgeldi. Türkgeldi is the crossbreed formed by crossbreeding of Kivircik with East Frisian. The bootstrap values are rather low (62%), indicating that these groups do not have strong support. In other words, while the breeds are highly differentiated from each other, the exact relationship between them cannot be established accurately. Indeed, when the NJ tree was constructed by using sheep samples individually (Figure 2), based on allele sharing distances, it was observed that individuals of the breeds do not form distinctly separate groups (details are not shown). Yet, it can be said that most of the groups represent one breed heavily (more than 50%). The branch nodes of those groups are shown in Figure 2. The shape of the tree, which is « star-like » (Figure 2), is typical for domestics [4, 10] and indicates the emergence of the breeds from relatively few founders followed by rapid increase in their size accompanied by selection, inbreeding and random genetic drift in the subsequent generations.

Name of the Locus		Breeds				
		Akkaraman	Awassi	Kıvırcık	Türkgeldi	Konya Merino
OarFCB20	Sample Size	35	35	35	34	35
	n_A	9	8	11	7	7
	H_e	0.7520	0.8318	0.8960	0.7713	0.7700
	H_o	0.6571	0.8065	0.8824	0.7813	0.7941
OarFCB304	Sample Size	35	35	35	34	35
	n_A	13	12	8	8	9
	H_e	0.8203	0.7282	0.6936	0.7911	0.7790
	H_o	0.8571	0.6774	0.4706	0.8485	0.6364
MAF65	Sample Size	35	35	35	34	35
	n_A	6	6	7	5	5
	H_e	0.7035	0.6653	0.7569	0.6150	0.6610
	H_o	0.6571	0.7097	0.800	0.7097	0.5714
Overall	MNA/locus	9.33	8.67	8.67	6.67	7
	Mean H_e/locus	0.7586	0.7418	0.7822	0.7258	0.7367
	Mean H_o/locus	0.7238	0.7312	0.7176	0.7798	0.6673
	Private allele	2	3	6	4	0

TABLE II. — Genetic diversity among breeds: number of alleles (n_a) observed per locus, observed heterozygosity (H_o) and expected heterozygosity (H_e) per locus, mean number of alleles (MNA) per locus, mean H_o and mean H_e values, and the number of private alleles observed for each breed.

Breeds	F_{IS}
Akkaraman	0.0465
Awassi	0.0145
Kıvırcık	0.0836
Türkgeldi	-0.0757
Konya Merino	0.0956

TABLE III. — F_{IS} (inbreeding coefficient) values of breeds averaged over 3 loci.

	Akkaraman	Awassi	Kıvırcık	Türkgeldi	Konya Merino
Akkaraman		0.064***	0.081***	0.097***	0.054***
İvesi	0.277		0.059***	0.118***	0.070***
Kıvırcık	0.399	0.272		0.085***	0.066***
Türkgeldi	0.408	0.501	0.371		0.141***
Konya Merino	0.233	0.289	0.301	0.634	

TABLE IV. — Pairwise differences between the breeds in terms of F_{ST} (upper tringle) and Nei's D_S (lower triangle).

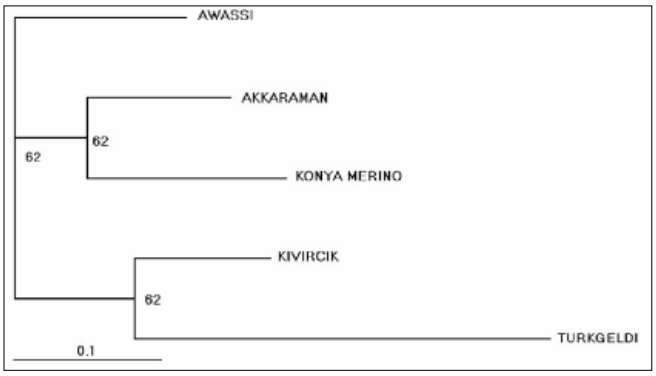

FIGURE 1. — Neighbour joining (NJ) tree of the breeds based on Nei's D_S. Bootsrap values were indicated on the nodes of the tree.

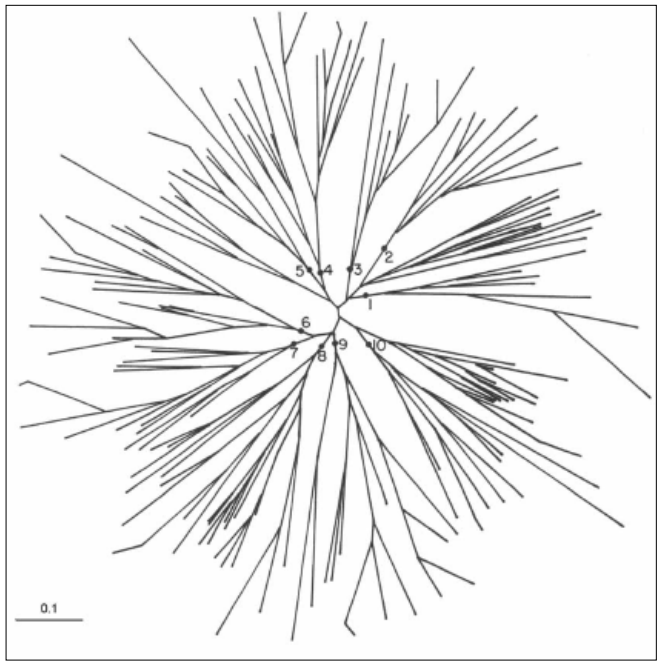

FIGURE 2. — Neighbour joining (NJ) tree of 174 individuals from 5 breeds based on allele sharing distances. The majority of the samples (≥50%) within the numbered branches belong to a single breed [1. Awassi (85.7%), 2. Awassi (62.5%), 3. Konya Merino (71.4%), 4. Kivircik (66.67%), 5. Konya Merino (88.89%), 6. Akkaraman (50%), 7. Türkgeldi (50%), 8. Türkgeldi (60%), 9. Akkaraman (50%) and 10. Kivircik (54.55%)].

Discussion

Genetic diversity of the breeds was determined by using n_A and H_e. Average n_A and H_e per breed indicated that the diversity is quite similar in all of the breeds. However, it can be noticed that crossbreeds, i.e. Türkgeldi and Konya Merino, exhibited slightly lower values than those of the native breeds with respect to both n_A and H_e.

Akkaraman, Awassi, Kivircik as well as Konya Merino and Türkgeldi are kept in large populations within agricultural or research enterprises. However, for the native breeds new individuals from the flocks owned by the local people were frequently introduced. The crossbreeds, in contrast, were bred within enterprises without any input from the outside. Therefore, crossbreds have relatively small populations compared to that of native breeds. This might be the reason of observing less genetic variability in crossbreeds than those of native breeds.

The genetic diversity levels of five Turkish breeds were compared with those of European breeds based on the same three microsatellites [10]. The breeds included in that study were both phenotypically and genotypically distant from each other and included Icelandic sheep, sheep from Greece and Cyprus (Chios), France, Germany, Hungary and also Awassi from Israel-Near East. Average n_A values per breed value varied between 4 and 9.3. There were two breeds exhibiting 9.3 and these were Awassi and Chios. Our native breeds had average n_A values of 9 for Akkaraman, 8.67 for Awassi and 8.67 for Kivircik. The average H_e values ranged between 0.60-0.77 in Bryne and collaborators' study [10] where 0.77 belonged to Awassi and the next highest was 0.76 (Racka, Hungary). In the present study, native breeds exhibited average H_e values as 0.78 (Kivircik), 0.76 (Akkaraman) and 0.74 (Awassi). Hence, it can be said that Turkish native breeds have high genetic diversities comparable with those of Awassi from Israel, Chios from Greece-Cyprus and Racka from Hungary. Furthermore, it must be noted that in BYRNE *et al.* study [10] sheep samples of Awassi were strictly unrelated. Especially, Chios samples were taken from different flocks within the mainland and from Cyprus. It can be said that, if the breeds of Turkey were represented by the individuals obtained from different flocks of the breed from diverse geographic areas, high (or the highest) genetic diversities that they posses would be observed more clearly. High genetic diversity within the breeds is one of the supporting observations for the closeness of the breeds to the centre of domestication [4, 20]. In conservation studies, one of the criteria to decide the priority of the breed is the high level of heterozygosity that it exhibits. In this study, even the three microsatellites revealed the preciousness of the Kivircik, Akkaraman and Awassi breeds. Private alleles observed within the study perhaps indicated the distinctness of Kivircik among the breeds considered. However, the number of private alleles observed must be examined in another study by employing individuals from all over the distribution

ranges of the breeds and with higher number of breeds throughout the world and with higher number of microsatellite loci.

Non-significant and almost uniformly positive F_{IS} values observed for the breeds (Table II) suggest that there is slight but non-significant inbreeding within the agricultural enterprises. Indeed, sizes of the populations in these enterprises are quite high; at least 1000 sometimes more than 5000. Hence, the level of inbreeding must be low.

Highly significant ($p<0.001$) differences between the breeds did not imply the presence of high inertia for the breeds (Figure 2). In other words, individuals of a single breed did not form a compact distinct group among themselves. In Byrne and collaborators' study [10] relatively more homogenous groups were observed in the NJ tree constructed by using the allele sharing distance. However, in that study 20 loci were used and breeds were geographically (and hence genetically) separated much more than that of the present study.

The assignment of an individual to a breed, with a high confidence based on its genotype, needs considerably higher number of loci with respect to the number of loci analysed for the present study. However, the three microsatellite loci was able to detect the presence of three groups formed by three breeds and their related crossbreeds (Figure 1). These three groups were (1) Awassi, (2) Akkaraman-Konya Merino, and (3) Kivircik-Türkgeldi.

As a conclusion, it can be said that the present study revealed the presence of a high degree of genetic diversity within the sheep breeds of Turkey. However, new studies employing higher number of loci, higher number of individuals from a wider geographic range of the breeds and higher number of breeds are necessary to understand the distinctness of the breeds.

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