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A Study on the Wool Characteristics of Several Turkish Sheep Breeds According to the Microsatellite DNA Types

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Abstract: This study was aimed to clarify the differences among the indigenous farm animal breeds by means microsatellite DNA polymorphism. The microsatellite DNA polymorphism in the Kıvırcık, Akkaraman, Awassi, Türkgeldi and Konya Merino sheep breeds were investigated. Repeated determined number of nucleotide as length of 1-6 nucleotide so called microsatellite are informative in phylogenetic studies. Three different microsatellite region of OarFCB304, OarFCB20, MAF65 were used in this study to compare the breeds genetically. The homozygote and heterozygote types of three mentioned loci were determined. The variation in the several wool characteristics of 5 native sheep breed investigated were examined according to the microsatellite DNA types. The relationship between several wool traits of material such as length, work to rupture, thickness, elongation, tenacity, etc and microsatellite DNA types were analysed.

Key words: Wool characteristics, sheep breeds, microsatellite, DNA types, PCR

INTRODUCTION

The averseness of importance of maintaining and monitoring farm animal genetic sources has increased over the last year. Many European countries including Turkey have developed national preservation and conservation programmers of their farm animal diversity.

In order to utilize and evaluate this kind of *in situ* and *ex situ* preservation study it is necessary to get support from molecular genetic levels of evidence. Selected sheep breeds needs to be characterized in order to clarify the potential of genetic diversity of sheep breeds. This study is aimed to clarify the differences among the indigenous farm animal breeds by means of microsatellite DNA polymorphisms. The microsatellite DNA polymorphisms in the Kıvırcık, Akkaraman, Ivesi, Türkgeldi and Konya Merino Sheep breeds were investigated.

Throughout the last 50 years a huge reduction in the genetic diversity of domestic animals has taken place. Rapid improvements in breeding techniques have resulted so called modern breeds with attractive high production in general. This process has also lead to decreasing a large number of locally adapted breeds have became extinct either through crossbreeding or via direct replacement with more favorable breeds[1,2].

By using microsatellite marker it is possible to verify the genetic diversity of sheep breeds and through that evaluate the level of which genetic variation is being lost or restructured in these population it is reported the there are more than. 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. In spite of different sheep breed may have similar rough phenotypic characterizes but still can be different genetically conversely breeds may look very different by genetically close related breeds which share the same alleles at similar frequencies are genetically close related whereas those having the same alleles at different frequencies or carrying many different alleles are genetically distant. For the analysis of genetic diversity in Turkish native sheep breeds we chose the analysis on microsatellite markers. The microsatellite markers is sequence of varying number of short tandem repeated sequences of 1-6 base pairs at a unique physical location in the genome which varies among individuals sufficiently to have its patterns of inheritance tracked through families. It is not possible conserves all genetics variations in all populations. The first step is to evaluate the genetic resources and the selection of appropriate populations for conservation. A strategy is than required that will maintain the widest possible level of genetic variation across the species[3].

The loss of indigenous breeds that having high resistance against many disease and tolerance to different environmental condition enable to exploit existence in

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more extreme environments also seriously effect the capacity of human society to live in large areas of world in sustainable manner without local breeds and traditional farming method which were used for keeping them many cultural aspects would disappear and the human population would lose a part of its own history and traditions.

There are a lot of molecular genetic techniques available in order to clarify the DNA polymorphism of breeds. They were consisted of mainly at this moment single nucleotide polymorphism, minisatellite, microsatellite, Variable Number Tandem Repeats (VNTR), Restriction Fragment Length Polymorphism called also (RFLP) and Random Amplified Polymorph DNA called shortly (RAPD)[4,5].

Microsatellite is stands for short repeated DNA sequences with length of 1-6 nucleotides in the whole genome. This was also called Short Tandem Repeats (STR). This type of polymorphisms can be used as an efficient tool for phylogenetic studies among breeds and level of inbreeding in population.

DNA fingerprinting takes advantage of the natural presence of repetitive regions of DNA sequence within genome. These regions of DNA do not contain gene that is they are non-coding. Some of repetitive regions are believed to play role in maintaining chromosome structure recombination and or regulatory control. Because these regions are non-coding they accumulate mutations faster then coding regions, where mutations are much more likely to affect individuals' survival and this tend not be transmitted to the next generation. Since mutation occurs randomly, each individual carries a unique of these repetitive sequences.

DNA fingerprinting is performed either by probing DNA with markers that control repetitive sequences on using PCR to amplify specific repeats regions within genome. Two types of DNA fingerprint techniques are performed in this concept. Variable Number Tandem Repeats (VNTR) analyses are PCR based methods of fingerprint are the relevant techniques in this area. VNTR's are classified as microsatellite (12-200 bases) and minisatellites (2-4 bases) in VNTR the regions of DNA that confirm different number of short repeating sequences in different individual and at different positions of genome (called loci) were analyzed. According to the existed phenomenal one person may live two repeats at each of three loci, another may live one copy at are locus and two at both of the others this the pattern will vary at third loci. In PCR based methods of fingerprinting identify unique profiles of DNA fragments by varying the nature of PCR primer and the conditions under which the primer anneals. The condition can be varied so that only sequences with exact complementarily to the primer sequence will be bind or at the other extreme, so that all sequence that are some what similar will bind. A profile of different sizes of bands is generated that reflects the various loci targeted by primers. PCR-based fingerprinting is typically used to screen whole genome producing large number of individual's specific bands. PCR based methods include randomly amplified polymorph DNA (RAPD) arbitrarily primed PCR (AP-PCR) and amplified length fragment polymorphism. The main reason the preference of microsatellite is the great variation existed by this type of polymorphism.

MATERIALS AND METHODS

DNA extraction and purification: Samples of genetic variations at three DNA microsatellites were investigated in three indigenous and two crossbreed Turkish sheep breeds. A sample of unrelated animals was studied in each of following breeds (the number of animals is indicated in parentheses) Kıvırcık (35), Akkaraman (35), İvesi (35), Türkgeldi (34), Konya Merinosu (35).

The loci were chosen on the basis of their location and distance in several chromosomes and no linkage criterion for synthetic markers. Microsatellites and their chromozomal location in parenthesis were as follows: OarFCB304 (Chr19), OarFCB20 (Chr2), MAF65(Chr15). Genomic DNA was extracted from whole blood samples by puncture of *V. jugulars* as 10 mL with antiquagilant tube. DNA isolations were made by the method so called fenol-cloroform techniques according to the Gündüz *et al.*[6] by using Proteinase-K digest. The blood samples were stored –20°C in deep freeze until isolation of DNA from samples.

Three different microsatellite regions of OarFCB304, OarFCB20, MAF65 were used in this study to compare the breeds genetically. The homozygote and heterozygote types of three mentioned loci were determined. The variation in the several wool characteristics of 5 native sheep breed investigated were investigated according to the microsatellites DNA types. The relationship between several wool traits of material such as length, work to rupture, thickness, elongation and tenacity and microsatellite DNA types were analyzed.

Details of the three microsatellites used are summarized in Table 1. All loci were autosomal with no evidence of any genetic linkage among the three microsatellites.

The PCR analyses were carried out using a Pelkin Elmer Thermal Cycles. Products of amplification were electrophoresed on 6% concentrated denaturing sequence gels (polyacrlyamid) and the bands of DNA

Table 1: Details of three sheep microsatellites DNA polymorphism loci used in the study

Name	Primers (5'-3')	PIC*	Annealing Temperature (°C)	Chromosome number	Accession number**	Reference
OARFCB304	P_1: CCCTAGGAGCTTT CAATAAAGAATCGG	0.54	63	19	L01535	Buchanan *et al.*[8].
	P_2: CGCTGCTGTCAACT GGGTCAG GG					
OARFCB20	P_1: GGAAAACCCCCAT ATATA CCTATA C	0.80	63	2	L2004	Buchanan *et al.*[8].
	P_2: AAATGTGTTTAAGA TTCCATACATGTGx	0.62	60	15	M67437	Buchanan *et al.*[8].
MAF65	P_1: AAAGGCCAGAGTAT GCAATTAGGA G					
	P_2: CCACTCCTCCTGA GAATAATATAACAG					

*PIC = Polymorphic Information Content, **Accession numbers are from Gene Bank

visualized by auto radiography as staining by silver nitrat($AgNO_3$). Microsatellite loci were amplified as follows. Genomic DNA (100 ng) was used as a template in total of 250 μL reaction mixture containing 200 μm dNTP each (1.0 μL), 2.0 mμ $MgCl_2$(2.0 μL), 1 X (TBE) buffer for PCR (2.5 μL); 2.5 μm forward and reversed primer (1.0 μL), Taq Polymeraz 1.5 units/reaction (0.3 μL) in distiller water of total 25.0 μL volume. Total reaction mixture volume was 25.0 μL and put in the (0.2) mL ependorf tube for PCR. PCR were carried out using a Pelkin Elmer thermo cycle with the following cycles; 5 min denaturation step at 94°C second step of total of (30) cycles of 1 min at 94°C denaturation, 1 min of 63°C annealing, 1 min of 72°C extension. The last step of amplification was again 1 cycle of 10 min at 72°C for extension. In order to avoid any contamination, application of negative control (not having any genomic DNA) were also conducted. Sizing of fragments was carried out with reference to standard fragments of 50 base pair length (fermentas). According to the resulted band resulted band diagram the homozygote (one band) and heterozygote (two bands) types were determined.

Several wool traits such as slenderness, tenacity, elongation, work to rupt, force, length of individuals were measured in the laboratory of Lalahan. Animal Breeding Research Institute of Ministry of Agriculture by using USTER UFDA 100 and USTER FL100 and FaFafegraph HrtME single Fiber Tensile Tester.

The possible meaning of variation observed in the data of wool traits according to the microsatellite polymorph types were investigated according to the Completely Randomized Experimental Design [7].

RESULTS AND DISCUSSION

The three microsatellites OARFCB304, OARFCB20, MAF65 generated 21, 14, 10 alleles, respectively from 174 individuals from 5 native and cross breeds sheep. Total 45 alleles were observed for three microsatellites. The variation observed in some wool traits according to the homozygote and heterozygote genotypes of three loci (Table 2). In order to investigate whether any relationship between microsatellite type and wool traits exist or not; the genotypes at each loci grouped as homozygote and heterozygote.

It is concluded that the possibilities of obtaining significant differences according to the genotypes of microsatellite were lower than $p<0.05$. So the wool traits differences between the phenotypic groups of microsatellite loci examined sourced mainly chance alone ($p>0.05$).

As it could be seen from the Table 2 the differences about the elongation measurements between heterozygote and homozygotes were 1.23% with respect of OARFCB 304 loci and not statistically important. The differences of two genotypes for force of wool (g), in the same loci, tenacity (g/den), work to rupt (g/cm), slenderness (micron) and length (cm) were also not significantly important in the mentioned loci. The results for the loci of ARFCB 20 and MAF 65 with respect of traits mentioned were also parallel with the first loci described (Table 3).

There were some researches showed that relationship between polymorphisms in the bovine lepton gene and beef carcass traits. It seems the relationship between microsatellite DNA types and several production traits would be long-range application. This idea includes mapping of QTL by linkage. Such mapping information if available particularly for those loci which affect the performance traits or disease resistance susceptibility can be used in breeding programs by either with in breed manipulation like marker assisted selection of young individuals on between breeds introgression programming[9].

It reported there are at least two loci that have shown association with resistance of Ruminants to gastro intestinal nematodes in ruminants. It is also reported an association between Single Nucleotide Polymorphism

Table 2: The distribution of wool traits according to the microsatellite loci genotypes

Loci	Génotype	Elongation (%)	Force (g)	Tenacity (g/den)	Work to rupt (g/cm)	Slenderness (micron)	Length (cm)
OARFCB304	Heterozygote	30.28±5.80	13.82±7.44	6.96±3.77	10.11±6.54	28.15±4.58	37.74±9.20
	Homozygote	29.05±6.72	13.49±7.085	6.75±3.52	9.75±6.11	28.56±4.56	36.53±8.68
	Total	29.91±6.10	13.72±7.33	6.89±3.70	10.00±6.41	28.27±4.57	37.38±9.04
OARFCB20	Heterozygote	29.92±6.07	13.46±7.18	6.75±3.60	9.82±6.62	28.10±4.46	37.23±9.20
	Homozygote	30.26±6.00	14.88±8.03	7.52±4.09	10.93±6.93	28.57±5.04	37.30±8.61
	Total	29.99±6.05	13.77±7.39	6.92±3.72	10.06±6.47	28.20±4.59	37.25±9.06
MAF65	Heterozygote	30.21±6.04	14.58±7.35	7.31±3.70	10.68±6.56	28.80±4.74	37.79±8.97
	Homozygote	29.81±5.76	12.78±7.09	6.46±3.61	9.31±6.03	27.53±4.20	37.30±10.53
	Total	30.09±5.95	14.03±7.31	7.05±3.70	10.26±6.43	28.41±4.61	37.64±9.47

Table 3: The distribution of genotypes at microsatellite loci of breeds examined

	OARFCB304						OARFCB20						MAF65					
	1	2	3	4	5	Σ	1	2	3	4	5	Σ	1	2	3	4	5	Σ
Heterozygote	5	10	18	5	12	50	12	6	4	7	7	36	12	9	7	9	12	49
Homozygote	30	31	16	28	21	116	23	25	30	25	27	130	23	22	28	22	16	111
Total	35	31	34	33	33	166	35	31	34	32	34	166	32	31	35	31	28	160

1:Akkaraman, 2:Awassi, 3:Kıvırcık, 4:Türkgeldi, 5: Konya Merino Sheep

(SNP) in the 5' region of growth hormone receptor gene and dairy production traits. Single Nucleotide Polymorphisms (SNP) in the 5' region of bovine growth hormone race in gene and its association with dairy production traits presented [10].

It reported there are at least two loci that have shown association with resistance of Ruminants to gastro intestinal nematodes in ruminants. It is also reported an association between Single Nucleotide Polymorphism (SNP) in the 5' region of growth hormone receptor gene and dairy production traits[11].

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