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Genetic Variation at Karayaka Sheep Herds Based on Random Amplified Polymorphic DNA Markers

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Abstract: Turkish Karayaka sheep breed that is one of the important native breed at Black Sea Region in Turkey was genotyped for 13 RAPD primers. Genotypes of 100 were collected from five different herds (BA, BF, LA, LD and KP herds) localized in the Samsun territory. The genetic similarity was obtained on the dendrograms individuals and herd. Within herds, individuals showed a similarity index between 0.857 to 0.420. The similarity index between populations ranged from 0.520 to 0.710. The binary results also were utilised by principal component analysis for comparison of herds and individuals. The genetic relationship of animals shows a difference among herds in terms of number of RAPD bands. These results show that some herds are effected possibly from other sheep breeds or reared purely such as KP.

Key words: Karayaka sheep breed, genetic similarity, RAPD

INTRODUCTION

Polymorphisms on the DNA level are frequently used as genetic markers for herd studies in domestic animals. Genetic markers and the variation detected at these loci reflect the level of variation in the entire genome. Using of genetic markers to introgress genes can shift specific characteristics of a population (Notter *et al*., 2007). RAPD assay which uses short oligonucleotide primers of arbitrary sequence to amplify genomic DNA by PCR enables an approach for identifying genetic markers (Cuswha *et al*., 1996; Williams *et al*., 1990). The random amplified Polymorphic DNA (RAPD) was utilised in population genetic studies in most species, including the various farm animals like cattle (Appannavar *et al*., 2003; Sharma *et al*., 2004), buffalo (Saifi *et al*., 2004) goat (Chen *et al*., 2001), chicken (Hwang *et al*., 2001; Okumus and Kaya, 2005) etc. The efficiency and usefulness of RAPD markers has been presented Cushwa and Medrano (1994) and demonstrated that RAPD genetic markers could be mapped to a specific locus in sheep (Cushwa and Medrano, 1994; Cushwa *et al*., 1996).

Domestication of sheep probably occurred around c. 9000 BC in Asia at the end of the mesolithic period (Ryder, 1984). Evolution forces of migration, mutation, selection and human activity has resulted in the vast array of lines and breeds that comprise domestic animal diversity. There has been an effort to document the origin, distribution, adaptability, morphological characteristics and performance of domestic animal resources (Dmitriev and Ernst, 1989; Simon and Buchenauer, 1993; Simon, 1990; Mason, 1996; Scherf, 2000). There are 629 sheep breeds currently maintained in the 52 European countries and 233 sheep breeds maintained in the 53 Asian and Pasific Countries (Scherf, 2000). In many developing countries, there are a number of indigenous herds that represent unique lines and to classify a large number of sheep is based on identifiable morphological characteristics with a local name (Shrestha, 2004). The new technologies enabled considerable advances to identify the landraces using the polymorphism.

Sheep is one of the most important domestic animals for Turkey which have 25 million head of sheep. The 24% of consumed meat is provided from the sheep (Kaymakcı *et al*., 2001). Karayaka sheep is a preferable breed for meat production and quality which is also well adapted to the warm and humid irrigated region of northcentral semi-arid region of Turkey (Ozcan, 1990; Akcapınar, 1994). However, it is generally found in middle Blacksea region. Purity of the breed is considered to be under constant risk due to increasing crossing with other breeds. This breed is generally found in Samsun, Amasya, Sinop, Ordu, Giresun and Tokat territories near to Black Sea Side. The herd size of this breed is about 833.000 individuals (Ozturk, 2001). This breed suffers from the unplanned crosses to increase fecundity (Olfaz *et al*.,

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2005). On the other hand, the genetic upgrading of the Karayaka sheep breed was performed several times with other sheep breeds (Olfaz, 1997; Olfaz *et al*., 2005). Although there a study on the sequencing base of Karayaka sheep breed by Meadows *et al*. (2007), information of this type has not been done using RAPD markers in the Karayaka breed. The individuals sampled closely related at least to the grandparent level and random selection of these individuals within a herd does not have pedigree information, regularly. However, it is known that the farmers, in some cases, add a ram to their herd from other breeds to improve them for fecundity or meat yield. In this study, we present the genetic variability and herd structure of Karayaka sheep by RAPD assay.

MATERIALS AND METHODS

A total of 100 blood samples were randomly collected from different herds including Bafra1 (BA), Bafra2 (BF), Kampus (KP), Ladik1 (LA) and Ladik2(LD) herds in 2004-2005 period. In the study, sampling of closely related animals was avoided. To ensure representative samples in the absence of pedigree information Karayaka sheep were collected from 5 farms. The number of selected individuals according to herd are presented by number in Table 1. Although the number of animals per flock and in total is important to allow useful conclusions to be drawn about population structure of the breed, the number of samples decreased about 30% due to the handicaps during the DNA extraction.

Genomic DNA samples was isolated using the standard phenol chloroform extraction method (Sambrook *et al*., 1989). DNA concentration was quantified with an Eppendorf Fluorometer and stock solutions were prepared and stored at -20°C. Dilutions of 10 ng μL^{-1} were stored at 4°C.

PCR reactions were performed in a 25 μL total volume containing 6.5 Ready Master Mix (SIGMA), 30 ng of genomic DNA, 30 pmol of primer (Operon Tech., Alameda, CA (OP)). A total of 120 decamer primers of arbitrary sequence were screened. Thirteen of the primers produced clearly informative bands. The number of RAPD markers have also an important phenomenon on the estimate of genetic diversity and changes between 6 to 93 decamer in the studies (Choy *et al*., 2001; Ramesha *et al*., 2002). The PCR were performed on an Hybaid thermal cycler starting initial denaturation time at 94°C (3 min), followed by 45 cycles of 94°C (1 min), 35°C (1 min) and 72°C (2 min) and final extension at 72°C (10 min). PCR products were detected by staining with ethidium bromide and visualized under UV light using SyngeneGel Documentation System. Molecular size of the RAPD polymorphisms were estimated with 100 bp DNA ladder (Amresco) for each gel.

Table 1: Selected animals taken place in the herds

Herds	Symbol	Animals
Bafra1	BA	1, 2, 3, 4, 5
Bafra2	BF	6, 7, 8, 9
Ladik1	LA	10, 11, 12, 13, 14, 15
Ladik2	LD	16, 17, 18, 19, 20, 21, 22, 23
Kampus	KP	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36

RAPD banding patterns were scored by the presence (1) and absence (0) for each sample and genetic similarity was calculated according to Jaccard (1908) using genetic similarity formula. The cluster analysis of genotypes was prepared using the similarity matrix by UPGMA method and principal component analysis using NTSYS computer programme, version 2.1 (Rohlf, 1989). The genetic difference between herds was calculated by chi-square for percent of polymorphic bands.

RESULTS

Genotypic data were obtained for a total of thirteen informative primers which were selected from 120 primers. Using these primers the parameters of DNA products per primer were summarized in Table 2. The number of DNA products per primer was 9 with a range of 6-12 bands. Primer OPC-11 and OPD-20 revealed one of the highest polymorphism among selected types. These primers can be considered as informative primers for selection in future. A total of 121 markers was scored for the thirteen primers and 51.2% of them were polymorphic. The size of the amplified fragments used in the study ranged from 121 to 2054 bp.

A dendrogram based on similarity calculated from 121 RAPD bands, did not segragate herds into groups (Fig. 1). The herd of KP was created from Bafra herd after at least ten cycles of selection and consequently, it is shown that Kampus herd is the same group with one of the Bafra herd. However, Ladik, (LD and LA) and Bafra (BA) herds are very distant from Kampus (KP) herd (Fig. 2). These large genetic distances between herds suggest that many loci are involved in the difference between Kampus and other herds that are likely polygenic. However, the similarity between herds in terms of number of bands was showed a significant difference of KP herd comparing other herds ($\chi^2 = 10.09$, $p<0.05$). The herd of KP showed higher genetic similarity than the other herds.

Using cluster analysis which is useful in identifying relationships among herds, many herds appeared in the dendrogram as discrete herds with individual genetic identity. It is very interesting that the herds, individually, showed with a similarity index between 0.857 to 0.420. From these results, this high level of genetic variability suggested that herds had been traded into these regions,

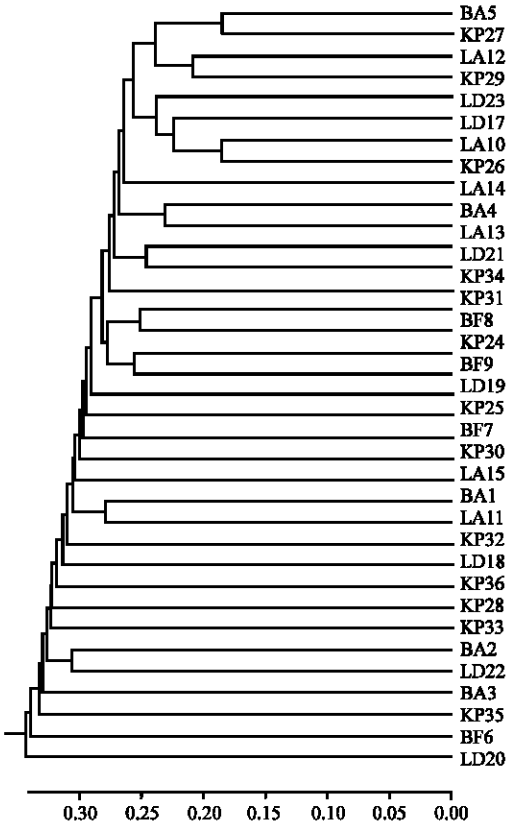

Fig. 1: Dendrogram showing the genetic similarity among 36 genotypes of Karayaka sheep breed

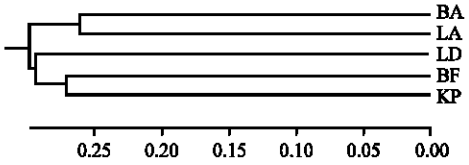

Fig. 2: Dendrogram showing the genetic similarity among 5 herd of Karayaka sheep breed

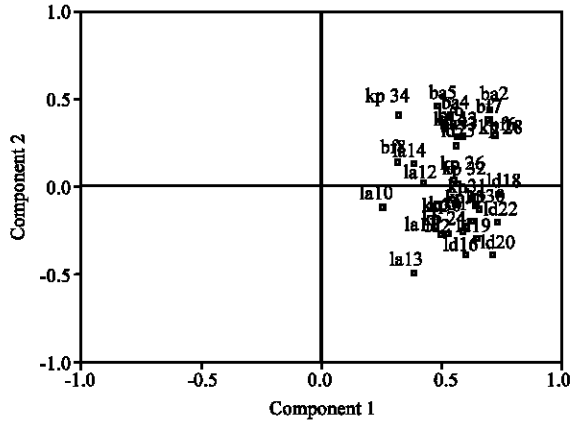

Fig. 3: Dendrogram showing the genetic similarity among 5 herd of Karayaka sheep breed

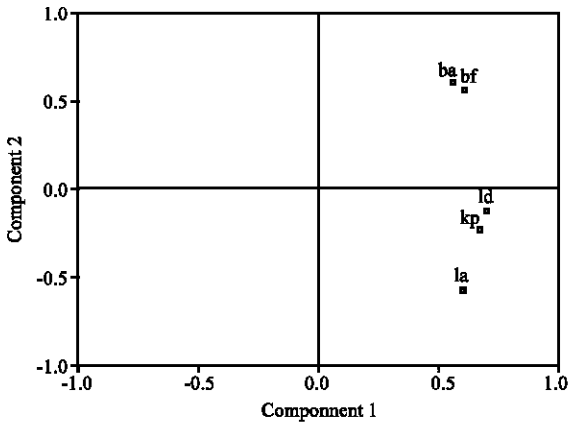

Fig. 4: Principal component analysis of herds

Table 2: Sequence number of total marker, number of polymorphic marker ans size of fragments (bp) of the thirteen selected RAPD primers

Primer	Sequence	No. of total marker	No. of polymorphic marker	Size of fragments (bp)
OPB-07	GGTGACGCAG	11	6	121-736
OPB-08	GTCCACACGG	8	4	636-2054
OPC-05	GATGACCGCC	8	5	449-1449
OPC-10	TGTCTGGGTG	10	3	360-1112
OPC-11	AAAGCTGCGG	12	5	389-1590
OPC-15	GACGGATCAG	6	3	678-973
OPD-02	GGACCCAACC	10	6	370-1372
OPD-18	GAGAGCCAAC	9	3	279-876
OPD-20	ACCCGGTCAC	12	5	572-1298
OPE-04	GTGACATCGC	8	4	448-1030
OPE-07	AGATGCAGCC	9	5	375-1090
OPE-15	ACGCACAACC	10	5	418-822
OPE-18	GGACTGCAGA	8	8	203-1052
Total\Range		121	62	121-2054

Table 3: No. of total and polymorphic markers among herd

Herds	No. total markers	No. polymorphic marker
Bafra1(5)	102	45
Bafra2(4)	106	44
Ladik1(6)	101	48
Ladik2 (8)	102	45
Kampus(13)	105	37

selected or migrated. The highest genetic similarity (0.857) was observed between numbered 7 and 28 genotypes which have been growth in distinct regions as Bafra2 (BF) and Kampus herds. The cluster analysis between populations supports these imlies (Fig. 2). Also, the similarity index between populations performed between 0.520 to 0.710.

The comparison of herds by the mean of band number was given in Table 3. The dendrogram of individual genotypes and among herds prepared by UPGMA was seen in Fig. 1 and 2, respectively. Principal Component Analysis (PCA) was demonstrated including all herds and loci in Fig. 3 and 4.

DISCUSSION

RAPD assay shows a difference among herds in terms of number of RAPD bands. These results show that some Karayaka herds can be effected from other breeds or are reared purely such as KP. Except OP-D20 (350 bp) primer in the selected primers, type A and B markers on the study done by Cushwa *et al.* (1996) were reproduced easily with multiplate (96 well-0.5 mL) and a Hybaid thermal cycler. The number and size of the fragments generated depends on the primer sequence used and on the source of the template DNA (Welsh *et al.*, 1991). The difference in the number of polymorphic markers among herds should be considered as the result of increasing or decreasing of homology by elimination of some genotypes, inbreeding of herd or attendance of individuals from different breeds to flock.

The number of scorable fragments per primer for sheep ranged between 1 to 12 (Cushwa *et al.*, 1996) due to the equation to predict the number of RAPD fragments used by Williams *et al.* (1993). Similar results were found in Karayaka sheep herds. On the other hand, Cushwa *et al.* (1996) reported identification of 85 RAPD polymorphisms from 131 RAPD primers within the international Mapping Flock reference population created with Coopworth, Merinoi Perendale, Romney and Texel breeds. In addition, polymorphic primers in Cushwa *et al.* (1996) exhibited similar results in the current study in terms of polymorphic marker size, with exceptions for OP-B7, OP-C10, OP-D18 and OP-E7 primers.

The scoring of RAPD polymorphisms in different pedigrees or herds is the homology of similarly sized fragments and a comparison of RAPD fingerprints provides supporting evidence for genetic similarity between different individuals (Hadrys *et al.*, 1992; Arnau *et al.*, 1994; Smith *et al.*, 1994).

The genetic similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers (RAPD) showed a similarity between 0.819 to 0.957 (Ali, 2003). This was due to the sampled individuals representing pure Egyptian breeds. Egyptian breeds showing a proximity between 0.957 to 0.913. In this study, the large amount of variation was observed between 0.857 to 0.420. The Karayaka breed has broad levels of genetic diversity which can be advantage for genetic improvement programs. Also, the variation between individuals reflect a diversity between herd by principle component analysis as seen in Fig. 3 and 4.

The focus on conservation and identification purpose can be suggested to be done by microsatellite markers because of labor intensive and co-dominant genotypic system of RAPD. The study done by RAPD markers gives an information on the genetic diversity and herd structure of the Karayaka sheep breed. On the other hand, it is known that Turkish Karayaka sheep breed has a characteristic from the angle of meat quality and adaptability for hard conditions. But it suffers from the body weight and litter size. The variation seen in this study shows that local herds of this breed can be yielded a higher homozygosity with selection by genetic improvement programmes.

This study demonstrates the usage of the RAPD assay for identifying polymorphic markers in sheep is useful for genetic similarity to compare the Karayaka sheep herds. Also, study provides a basis for ensuring that any future conservation, utilization and genetic improvement programmes. The larger genetic variation can be useful for breeding programme carrying useful genes in future. In this study, it seems that Turkish Karayaka sheep breed herds have an affect under genetic flow from other breeds or the source of variation comes from wild type characteristics. On the other hand, it is considered that large diversity can be resulted whether from unplanned cross-breeding or local variations belong to Karayaka breed possibly. To see the gene flow of herds, the study should continue with other Turkish breeds such as Sakiz, Sonmez etc. taken place in the region by crossing. This study is interesting because of the large variation and has also, an importance from the angle of genetic conservation of breed.

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