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MITOCHONDRIAL DNA (mtDNA) HAPLOGROUP COMPOSITIONS OF THREE NATIVE TURKISH SHEEP BREEDS AND THEIR IMPLICATIONS ON THE CONSERVATION STUDIES

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ABSTRACT

Sheep domestication is believed to have occurred at least by three separate events in different domestication centers. Archeological studies indicated that Turkey might be harboring the earliest one and is close to the other suggested centers. Studies involving some of the Turkish sheep breeds revealed that they posses high genetic diversity, indicating their prime importance in conservation studies. Yet, which one(s) must be conserved? Studies on the evolutionary history of breeds may help to resolve different causes of high diversity in Turkish breeds and thereby help to construct sound conservation strategies for native sheep breeds in Turkey. In this line, in the present study, composition of sheep mtDNA haplogroups indicating different domestication events, among three native breeds, as a part of a national project TURKHAYGEN-I, was determined and results were evaluated comparatively with those available from the literature.

AIMS OF THE STUDY

→to compare the two different methods used for the mtDNA haplogroup typing.
→to evaluate the robustness of the results concerning mtDNA haplogroup frequencies of the breeds.

INTRODUCTION

→In the study of Hiendleder *et al*. (1998), two distinct mtDNA haplogroups: A and B were observed. Pedrosa *et al*. (2005) and Bruford and Towsend, (2006) observed another distinct haplogroup: C. Thus mtDNA haplogroup number was raised to three.

→Generally, presence of distinct mtDNA haplogroups are regarded as the products of different domestication events (Luikart *et al*., 2001). Hence, for the sheep at least three domestication events can be assumed.

→Haplogroup A (HPG-A) can be called as Asian Lineage, similarly HPG-B as European Lineage and HPG-C as South Asian Lineage because of their distribution patterns (Bruford and Towsend, 2006).

→Studies of Tapio *et al*. (2006) and Meadows *et al.* (2007), revealed two more haplogroups. These haplogroups have been rarely seen in Eurasia but were encountered in Turkish domestic sheep breeds (Pedrosa *et al*., 2005; Meadows *et al*., 2007 and Koban *et al*., 2008). They are called D and E haplogroups.

→On the other hand, archeological studies indicated that there must be at least three domestication events which took place in separate domestication centers. The oldest one is the Nevali Çori, in Turkey. Second one is the Zagros Mountains in the Border of Iran, Iraq and Turkey. For the third one: Indus Basin, India/ Israel/North of Caspian Sea are suggested places (Pedrosa *et al.,* 2005) and they are close to Turkey.

→So Turkey is expected to have high genetic diversity as the signature for maintained diversity since the earliest domestication and due to the admixture of domestication products. Indeed it is exhibiting high genetic diversity (Bruford *et al*., 2003; Uzunhan *et al*., 2006; Lawson-Handley *et al*., 2007 and Peter *et al*., 2007).

→Furthermore, domestic sheep spread from Turkey to Europe. (Clutton-Brock, 1981) During this spread genetic information must be lost. Some of these might be important and still existing in native Turkish sheep breeds.

→So Turkish sheep breeds, because they exhibit high genetic diversity and may harbor ancestors of European sheep, might have prime importance in conservation studies.

→Yet, native Turkish sheep breeds are under threat (Longworth, 2005).

→Urgently, their genetic structure must be studied and the results must be evaluated comparatively.

→Since mtDNA haplogroups are indicative of domestication events and since single strand conformational polymorphism (SSCP) of ND4 region and restriction fragment length polymorphism (RFLP) of control (CR) region on the mtDNA were employed in different studies (Guo *et al*., 2006; Bruford and Towsend, 2006), compatibility of their results will be tested in the present study.

→Furthermore, reproducibility of the results of the genetic studies must be tested before taking any action towards conservation.

MATERIALS METHODS

→Three breeds: **Karayaka** (from Ordu and Tokat provinces in Black Sea Region); **Akkaraman** (from Konya province in Central Anatolian Region); **Gökçeada** (from an Aegean Island near to Çanakkale in Marmara Region) were sampled.

→50 individuals as at most 2-3 old individuals/ flocks and at least 10 flocks were sampled for each breed.

→DNA samples were isolated from blood by phenol chloroform method. (Sambrook *et al*., 1989)

→CR and ND4 region of mtDNA were amplified by Polymerase Chain Reaction (PCR).

→CR was analyzed by RFLP to identify the haplogroups. *NsiI* (*Mph1103I/AvaIII*) enzyme was used for digestion of PCR products. (Bruford and Towsend, 2006)

→ND4 region was analyzed by SSCP to identify the haplogroups. (Guo *et al*., 2006)

→Statistical analyses were performed with Minitab 13 package program. (Minitab Inc.)

RESULTS

→CR and ND4 region's amplification products by PCR are illustrated in Figures 1 and 2.

→CR amplification product, when treated with enzyme *NsiI*, differentiates haplogroups as A, B and B/C, as described by Bruford *et al*. (2006). A CR RFLP result was displayed in Figure 3.

→ In accordance with Guo *et al*.'s (2006) study, with the help of SSCP, ND4 region of mtDNA can differentiate haplogroups as A, B and C. A result from the present study was displayed in Figure 4.

→Results of two methods, that were used for haplogroup determination, is %100 same for Karayaka, %98 same for Gökçeada and % 94 same for Akkaraman. (%97.3 in overall, total n=150.) One individual from Gökçeada showed different haplogroups with respect to different methods (RLFP-B Haplogroup, SSCP-C Haplogroup). Two individuals from Akkaraman gave unidentified but different SSCP bands, but they showed B/C haplogroup with RFLP method and one individual from Akkaraman gave unidentified RFLP banding pattern while it showed A haplogroup with SSCP band. Unidentified bands are displayed in Figures 5 and 6.

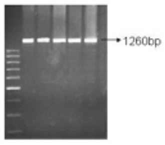

Figure1: PCR results of CR region.

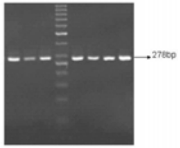

Figure 2: PCR results of ND4 region.

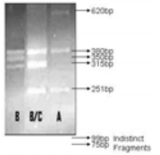

Figure 3: RFLP results of CR region.

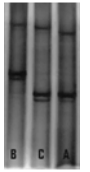

Figure 4: SSCP results of ND4 region.

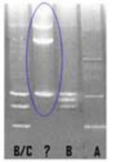

Figure 5: Identified and unidentified RFLP patterns.

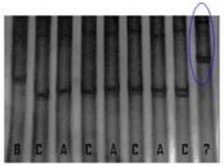

Figure 6: Identified and unidentified SSCP patterns.

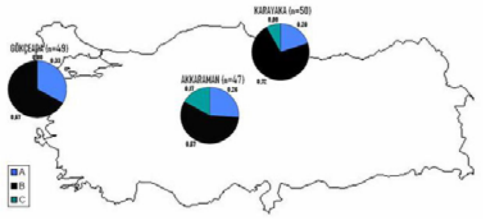

Figure 7: Allele frequencies of haplogroups in sheep breeds. (Unidentified/unmatched results were not taken into consideration while calculating allele frequencies.)

RESULTS

→Results are compared with the results of Koban *et al*., (2008)'s study and given in Figure 8. Two of the breeds were common between the two studies. Individuals (n~50 in both of the studies) were independently selected and by chance different flocks were sampled in these two studies.

→Karayaka breed was also analyzed in two other previous studies (Meadows *et al*., 2007; Pedrosa *et al*., 2005). When the results were compared, it has seen that there is no HPG-C observed in Karayaka in one study (Meadows *et al*., 2007), and HPG-C was observed with a 35% frequency in the other study (Pedrosa *et al*., 2005). The HPG frequency pie charts as observed in Karayaka in both studies are given in Figure 9.

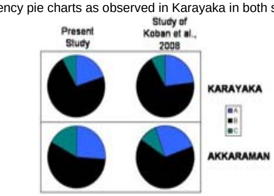

Figure 8: Allele frequency comparison of results with this study and the study of Koban *et al.* (2008) Difference between the results are not significant (p>0.05) by chi-square test.

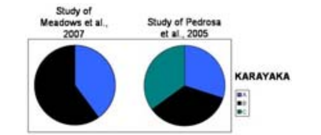

Figure 9: Allele frequency comparison of results with the study of Pedrosa *et al*., 2005 and Meadows *et al*., 2007. Difference between the results are significant (p<0.05) by chi-square test.

DISCUSSION AND CONCLUSIONS

→Based on the two methods (CR Region/RFLP and ND4 Region/SSCP), obtained mtDNA HPG results were same by 97.3% of the comparisons. Hence, results based on two different methods can be pooled together. Yet the presence of ~ 3% discrepancy between them must be remembered.

→The two different haplogroups observed by one Gökçeada individual or presence of unidentifiable patterns in SSCP or RFLP can be due to the presence of mutations in pattern determining sites of the two mtDNA regions.

→Individuals which gave unidentified band patterns can be possessing D or E haplogroup. These individuals will be sequenced to determine their haplogroups. Results may help to find a pattern for these rare HPGs.

→There is no significant difference between allele frequencies in this study and those of Koban *et al*.(2008)'s. This mainly shows that sampling in the studies is such that breeds are well represented in both of the studies.

→However, in two other studies (Pedrosa *et al*., 2005 and Meadows *et al*., 2007) where Karayaka from the present study was common, sampling strategy and hence the HPG frequencies were significantly (p<0.05) different.

→Since Turkish native breeds harbor all 3 HPGs (Pedrosa *et al*., 2005; Meadows *et al*., 2007; Koban et *al*., 2008 and present study) it can be concluded that native breeds are mixtures of 3 domestication products.

→The ones, for instance Karayaka, possessing highest European lineage (HPG-B) frequency might be an ancestral breed of European breeds. Therefore may deserve a high priority of conservation among the native breeds.

→Yet, present study indicated that results of genetic studies must be reproducible before any action to be taken in further studies. Sampling strategy and sample sizes must be carefully chosen to accurately represent the breeds.

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