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# GENETIC DIVERSITY OF KARYA AND ÇINE ÇAPARI SHEEP

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***Abstract***

*Genetic diversity of Karya (n=117) and Çine Çapari sheep (n=123), which is indigenous breed of Turkey, were investigated by 10 ovine microsatellite markers proposed by the Food and Agriculture Organization (FAO). A total of 105 and 115 were observed in Karya and Çine Çapari sheep breed respectively in this study. A wide range of genetic variability was observed as allele number from 7 (OarCP34) to 14 (DYMS1) for Çine Çapari and 6 (OarCP34) to 14 (OARJMP58) in Karya sheep breed. The estimated observed heterozygosities (Ho) were between 0.450 and 0.950 in Çine Çapari and 0.541 and 0.841 in Karya sheep breed. The highest genetic identity (0.8131) was observed between Karya and Çine Çapari sheep breed. The results obtained in the present study will help to interpret the genetic structure of indigenous Karya and Çine Çapari sheep.*

***Key words:*** *Çine Çapari sheep, genetic diversity, Karya sheep, microsatellite, indigenous sheep.*

## INTRODUCTION

Native breeds are the primary elements of animal breeding and they have complied with ecological, social and economical conditions of different geographies. These elements have taken shape within the process of thousands of year's agricultural society throughout history of humanity. Together with industrialization, sociological and economical conditions rapidly changing have introduced the necessity of benefiting more from native breeds. Statistical methods in breeding studies done especially in livestock over the past century have found a common field of application (Beuzen and et al. 2000). Thanks to rapid improvement in molecular biology today, base sequence of DNA can be defined, the place of the genes can be determined, the relationships between the genes can be examined and some genes can be transferred from living creature to living creature. As it's in the other species, molecular genetics methods for identification of genetic structure in livestock have developed rapidly in recent years and the usage of the methods has become widespread (Arranz et al. 1998, Baumung et al. 2004, Pariset et al. 2003, Montaldo, 1998). Specific microsatellite genetic markers to DNA areas are commonly used to identify genetic variety in animals (Bruford et al., 1996).

Çine Çapari sheep breed are raised in highlands of Aydin province. It has been localized in the borders of Aydin province. With so many scientific study done by Adnan Menderes University, the properties of the breed, its current condition are introduced and it's suggested that it's at risk as genetic resource and the breed is taken under conservation (Karaca et al., 1999a,b,c; Karaca et al., 2004; Karaca and Cemal, 2005; Binbas and Cemal, 2007). Karya sheep has developed in consequence of systemless back-crossing of Sakiz, Kivircik or Sakiz x Kivircik cross-bred rams with native breeds (Ödemis, Çine Çapari, Dagliç etc.) in Western Anatolia in the last 20 years period by animal breeders. Since Karya which is a genotype having high reproductive performance and milk yield is preferred by animal breeders, it has become widespread in Western Anatolia in recent years (Karaca et al. 2009). The purpose of this study is to determine the genetic diversity in DNA level of Karya sheep that they are commonly raised in Western Anatolia day by day and Çine Çapari which is under the threat of extinction by microsatellite DNA (STR, SSR) markers.

## MATERIALS AND METHODS

Obtaining DNA sample from two hundred and forty animals from Karya (N=117) and Çine Çapari sheep (N=123) (Figure 1) were genotyped with 10 microsatellite markers that selected from the list recommended by FAO (2004). Three multiplex groups were formed with 8 out of the 10 microsatellites. Annealing temperatures of MAF65 and DYMS1 were not appropriate for the other 3 multiplex groups. Therefore, these two microsatellites were amplified by Polymerase Chain Reaction (PCR) separately. Table 1 shows details for the considered microsatellites.

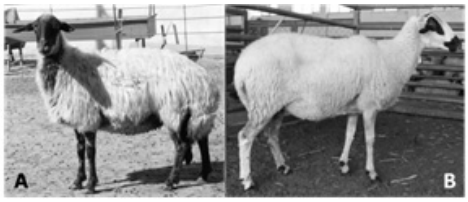


Figure 1. Çine Çapari (A) and Karya sheep (B)

Table 1. Details of considered microsatellites

| Locus Name | Primers | Base Pair (bp) |
|---|---|---|
| OarCP34 | **F:** GCTGAACAATGTGATATGTTCAGG<br>**R:** GGGACAATACTGTCTTAGATGCTGC | 112-130 |
| OarFCB304 | **F:** CCCTAGGAGCTTTCAATAAAGAATCGG<br>**R:** CGCTGCTGTCAACTGGGTCAGGG | 150-188 |
| OarFCB193 | **F:** TTCATCTCAGACTGGGATTCAGAAAGGC<br>**R:** GCTTGGAAATAACCCTCCTGCATCCC | 96-136 |
| OarJMP29 | **F:**GTATACACGTGGACACCGCTTTGTAC<br>**R:** GAAGTGGCAAGATTCAGAGGGGAAG | 96-150 |
| OarFCB128 | **F:** ATTAAAGCATCTTCTCTTTATTTCCTCGC<br>**R:** CAGCTGAGCAACTAAGACATACATGCG | 96-130 |
| BM8125 | **F:** CTCTATCTGTGGAAAAGGTGGG<br>**R:** GGGGGTTAGACTTCAACATACG | 116-122 |
| OarJMP58 | **F:** GAAGTCATTGAGGGGTCGCTAACC<br>**R:** CTTCATGTTCACAGGACTTTCTCTG | 145-169 |
| OarVH72 | **F:** GGCCTCTCAAGGGGCAAGAGCAGG<br>**R:** CTCTAGAGGATCTGGAATGCAAAGCTC | 121-135 |
| DYMS1 | **F:** AACAACATCAAACAGTAAGAG<br>**R:** CATAGTAACAGATCTTCCTACA | 159-211 |
| MAF65 | **F:** AAAGGCCAGAGTATGCAATTAGGAG<br>**R:** CCACTCCTCCTGAGAATATAACATG | 123-135 |

DNA was isolated from blood samples using a DNA extraction kit. Specific genomic regions were amplified by Polymerase Chain Reaction (PCR) in accordance with the touchdown PCR technique. The thermal cycling conditions are given in the Table 2.

For every microsatellite locus, the amplification reaction took place in a total volume of 25 µl and contained the following constituents in the final concentrations indicated in brackets; dNTP's (0·2 mM for each one), MgCl2 (2.0 mM), primers (0.25 mM for each one), and Taq DNA polymerase (1 unit reaction–1). Approximately 100 ng of genomic DNA was used as template for each of PCR amplification. Fragmentanalysis was achieved using the Beckman Coulter CEQ 8000 Genetic Analysis System. Obtained data was analyzed by the Beckman Coulter CEQ Fragment Analysis Software.

Table 2. Thermal cycling conditions according to Touchdown PCR

| Loci | Denaturation (°C) | Annealing (°C) | Extension (°C) |
|---|---|---|---|
| OarCP34 | 95<br>*45 sec* | 60-58<br>*45 sec* | 72<br>*45 sec* |
| OarFCB193 | | | |
| OarFCB304 | | | |
| OarJMP29 | 95<br>*45 sec* | 61-57<br>*45 sec* | 72<br>*45 sec* |
| OarFCB128 | | | |
| BM8125 | | | |
| OarJMP58 | 95<br>*45sec* | 60-56<br>*45 sec* | 72<br>*45 sec* |
| OarVH72 | | | |
| MAF65 | 95<br>*45sec* | 59-57<br>*45 sec* | 72<br>*45 sec* |
| DYMS1 | 95<br>*45sec* | 52-50<br>*45 sec* | 72<br>*45 sec* |

The data were analyzed using GenAlEx (Peakall and Smouse, 2006), PowerStatsV12 (Brenner and Morris, 1990), MEGA 4 (Tamura et al., 2007), Arlequin 3.5 (Excoffier and Lischer, 2010) and POPGENE (Yeh et al., 1997) softwares. A dendrogram based on Nei′s (1978) genetic distances was obtained using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method.

## RESULTS AND DISCUSSIONS

In the study, 105 allele in Karya sheep and 115 in Çine Çapari sheep from 10 microsatellite locus has been observed. Average allele number in a locus has been respectively 11.5 and 10.5 in Karya and Çine Çapari sheep. The results obtained are in compliance with the literature (Cemal et al., 2013; Yilmaz and Karaca, 2012). The locus which has the highest allele has respectively appeared as OarFCB193 (17) and OarJMP58 (14) in Karya and Çine Çapari sheep. The locus which shows the lowest polymorphism in Karya and Çine Çapari sheep has been OarCP34. The significant differences in allele numbers in populations indicates to high genetic diversity (Table 3).

Table 3. ASR (bp), nA, nE, Ho, He and PIC values in considered microsatellites

| Loci | ÇINE ÇAPARI | | | | | | | KARYA | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | ASR | na | ne | Ho | He | PIC | N | ASR | na | ne | Ho | He | PIC |
| OarCP34 | 121 | 112-124 | 7 | 4.48 | 0.843 | 0.777 | 0.74 | 113 | 112-122 | 6 | 4.25 | 0.841 | 0.765 | 0.74 |
| OarFCB193 | 121 | 96-136 | 17 | 8.67 | 0.950 | 0.885 | 0.87 | 115 | 96-140 | 13 | 3.32 | 0.774 | 0.699 | 0.69 |
| OarFCB304 | 121 | 160-190 | 11 | 3.80 | 0.727 | 0.737 | 0.70 | 115 | 148-188 | 13 | 5.44 | 0.800 | 0.816 | 0.77 |
| OarJMP29 | 122 | 116-156 | 13 | 4.29 | 0.820 | 0.767 | 0.74 | 113 | 110-158 | 13 | 4.88 | 0.770 | 0.795 | 0.78 |
| OarFCB128 | 117 | 100-128 | 10 | 4.86 | 0.692 | 0.794 | 0.77 | 111 | 100-134 | 11 | 3.90 | 0.541 | 0.744 | 0.74 |
| BM8125 | 119 | 108-138 | 13 | 4.78 | 0.782 | 0.791 | 0.76 | 113 | 108-130 | 8 | 3.35 | 0.726 | 0.702 | 0.68 |
| OarJMP58 | 122 | 141-171 | 13 | 4.32 | 0.713 | 0.769 | 0.74 | 115 | 143-169 | 14 | 5.92 | 0.748 | 0.831 | 0.82 |
| OarVH72 | 122 | 123-143 | 8 | 3.88 | 0.730 | 0.742 | 0.71 | 114 | 123-139 | 9 | 5.89 | 0.798 | 0.830 | 0.81 |
| MAF65 | 111 | 125-141 | 9 | 4.38 | 0.568 | 0.772 | 0.74 | 113 | 121-139 | 7 | 3.44 | 0.823 | 0.709 | 0.66 |
| DYMS1 | 120 | 169-201 | 14 | 4.31 | 0.450 | 0.768 | 0.74 | 114 | 181-203 | 11 | 5.13 | 0.763 | 0.805 | 0.78 |
| **Mean** | | | **11.50** | **4.78** | **0.73** | **0.78** | **0.75** | | | **10.50** | **4.55** | **0.76** | **0.77** | **0.75** |
| **St.dev.** | | | **3.064** | **1.407** | **0.141** | **0.041** | **0.048** | | | **2.838** | **1.033** | **0.084** | **0.053** | **0.055** |

Observed heterozygosity values obtained from Karya sheep are in the given range (Yilmaz and Karaca, 2012; Grigaliunaite et al., 2003, Tascon et al., 2000). Expected heterozygosis values obtained from Çine Çaparı sheeps has been higher than the studies made with the other sheep breeds (Tapio et al., 2005; Handley et al., 2007; Pramod et al., 2009; Tascon et al., 2000; Grigaliunaite et al., 2003 ). This situation can be explained with the high level of polymorphic information content of locus studied. Genetic similarity and genetic distance between Çine Çaparı and Karya sheep have been given in Table 4. Dendrogram belonging to genetic distance between populations studied has also been given in Figure 2.

Table 4. Genetic similarity (above diagonal) and genetic distance (below diagonal) between Çine Çaparı and Karya sheep

| | Çine Çaparı | Karya |
|---|---|---|
| Çine Çaparı | ****** | 0.8131 |
| Karya | 0.2069 | ****** |

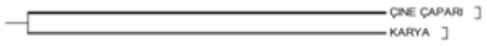


Figure 2. Dendrogram based on Nei's genetic distances between Çine Çaparı and Karya sheep

Above diagonal values present in Table 4 gives the genetic similarities of the breeds. As it's understood from the table, there has been a genetic similarity in high level between the populations. It has been suggested that Çine Çaparı sheep can provide contribution to Karya sheep (Karaca et al., 1999a; Karaca et al., 2004; Karaca and Cemal, 2005; Karaca et al., 2009). When this situation is taken into consideration, obtainment of these genetic similarity values is seen as a normal result. Factorial Correspondence Analysis (FCA) graphic has been drawn to show how the individuals in population are separated. The results obtained are given in the following multidimensional platform (Figure 3).

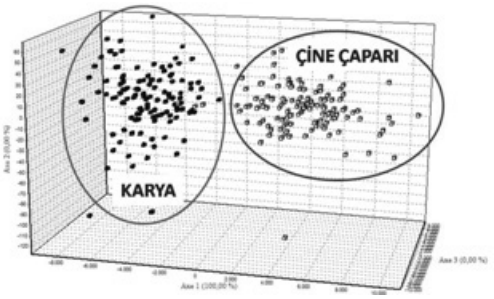


Figure 3. Factorial correspondence analysis (FCA) of Cine Capari and Karya sheep

When FCA graphic (Figure 2) has been examined, it's mentioned that the individuals in Çine Çaparı and Karya sheep populations constitute a group between each other. Dense clustering in Çine Çaparı and Karya sheep populations can be observed or there can be animals which remain between two clusters or enter into the other cluster. This result obtained shows the necessity of maximum microsatellite locus usage in characterization.

If the population performs specific assumptions, simple mathematical engagement which is known as Hardy- Weinberg law is in question to calculate genotype frequencies from allele frequencies. The information obtained from 10 microsatellite locus has been determined by using $\chi^2$ test in terms of suitability for Hardy-Weinberg equilibrium and it's given in Table 5.

Table 5. Chi-Square test values belong to 10 microsatellites in all population

| Loci | Çine Çapari Sheep | | | | Karya Sheep | | | |
|---|---|---|---|---|---|---|---|---|
| | DF | $X^2$ | Prob | Sign | DF | $X^2$ | Prob | Sign |
| OARCP34 | 21 | 20,96 | 0,461 | NS | 15 | 12,326 | 0,654 | NS |
| OARFCB193 | 136 | 180,51 | 0,006 | ** | 78 | 37,164 | 1,000 | NS |
| OARFCB304 | 55 | 141,41 | 0,000 | *** | 78 | 115,917 | 0,003 | ** |
| OARJMP29 | 78 | 308,40 | 0,000 | *** | 78 | 81,850 | 0,361 | NS |
| OARFCB128 | 45 | 138,66 | 0,000 | *** | 55 | 152,422 | 0,000 | *** |
| BM8125 | 78 | 216,77 | 0,000 | *** | 28 | 15,923 | 0,967 | NS |
| OARJMP58 | 78 | 254,78 | 0,000 | *** | 91 | 238,865 | 0,000 | *** |
| OARVH72 | 28 | 26,87 | 0,525 | NS | 36 | 88,909 | 0,000 | *** |
| MAF65 | 36 | 183,45 | 0,000 | *** | 21 | 16,580 | 0,736 | NS |
| DYMS1 | 91 | 365,78 | 0,000 | *** | 55 | 44,779 | 0,836 | NS |

*** P< 0.001; ** P< 0.05

When the results of ki-square ($X^2$) test made in terms of suitability for Hardy-Weinberg equilibrium on the basis of population are evaluated, it's seen that 8 locus in Çine Çapari population and 4 locus in Karya population aren't in Hardy-Weinberg equilibrium. When it's considered that these locus given in Table 5 aren't in Hardy-Weinberg equilibrium and there are selection studies carried out in Karya population and protection program applied in Çine Çapari sheep, it's seen as a normal situation. These findings are in compliance with the findings informed by Yilmaz and Karaca (2012) and Cemal et al. (2013).

## CONCLUSIONS

In this study, genetic diversity of Karya sheep, which have high reproductive performance and milk yield has been preferred by breeders in the western Anatolia region, and Çine Çapari sheep, which is under conservation indigenous breed of Turkey, have been determined by using 10 ovine microsatellite markers proposed by the Food and Agriculture Organization (FAO) in DNA level.

The results obtained have shown that there is high level of genetic similarity between Karya and Çine Çapari sheep populations. In addition to this, in this study it's suggested that genetic diversity in current gene pool belonging to these two breeds is significantly high. In Western Anatolia, in sheep genotypes over the last 20-30 years, there is a change with the effect of consumer demands. This situation has enabled fat tail breeds such as Ödemis, Çine Çapari and Dagliç, especially present in Western Anatolia to turn into a form which has thin tail by cross-breeding with Sakiz, Kivircik or Sakiz x Kivircik cross-breed rams (Karaca and Cemal, 1998, Karaca and Cemal, 2005, Karaca et al., 2009). The information obtained in the meaning of genetic similarity supports scientific information suggested by previous phenotypic methods.

Consequently, the findings obtained from this study have provided significant contribution to the literature as being the first study regarding identification of two breeds in molecular level.

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## REFERENCES

Arranz, J.J., Bayo´n, Y., San Primitivo, F., 1998. Genetic relationships among Spanish sheep using microsatellites. Anim. Genet., 29, 435–440.

Baumung, R., Simianer, H., Hoffmann, I., 2004. Genetic diversity studies in farm animals − A survey. J. Anim. Breed. Genet., 121, 361–373.

Beuzen, N.D., Stear, M.J., Chang, K.C., 2000. Review molecular markers and their use in animal breeding. Vet J, 16, 42-52.

Binbas, P., Cemal, I., 2007. Yerli Gen Kaynagi Çine Çapari Koyunlarda Genetik Çesitliligin RAPD Belirteçleri ile Belirlenmesi. 5. Ulusal Zootekni Bilim Kongresi, Poster Bildiri, 5-8 Eylül 2007, Yüzüncü Yil Üniversitesi, Van

Brenner, C., Morris, J., 1990. Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. Proceedings for the 1th International Symposium on Human Identification. Promega Corporation, Madison, Nov. 1989 pp. 21-53.

Bruford, M.W., Chessman, D.J., Coote, T., Gren, H.A.A., Haines, S.A., O'Ryan, C., Williams, T.R. 1996. Microsatelites and their application to conservation genetics. Molecular Genetic Approaches in Conservation. Oxford University Pres, 278-297

Cemal, I., Yilmaz, O., Karaca, O., Binbas, P., Ata, N., 2013. Analysis of genetic diversity in indigenous Çine Çapari sheep under conservation by microsatellite markers. Kafkas Üniversitesi Veteriner Fakültesi Dergisi.19(3):383-390, DOI: 10.9775/kvfd.2012.7857

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour, 10, 564-567.

FAO, 2004. Food and agriculture organisation of the United Nations secondary guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD) : recommended microsatellite markers.http://www.fao.org/docrep/014/i2413e/i2413e00.pdf

Grigaliunaite, I., Tapio, M., Viinalas, H., Grislis, Z., Kantanen, J., Miceikiene,I., 2003. Microsatellite variation in the Baltic sheep breeds. Vet Med Zoot, 21, 66-73.

Karaca, O., Cemal, I., 1998. Bati Anadolu koyunculugunda genetik kaynaklarin korunma ve kullanimi. Ege Bölgesi 1.Tarim Kongresi, s.573-582, 7-11 Eylül 1998, ADÜ Ziraat Fakültesi, Aydin.

Karaca, O., Çetiner, S. ve Cemal, I., 1999a. Çine Çapari koyunlarin kimi özellikleri ve genetik kaynak olarak korunmasi olanaklari. Uluslararasi Hayvancilik'99 Kongresi, s.558-563, 21-24 Eylül 1999, Ege Üniversitesi Ziraat Fakültesi, Izmir.

Karaca, O., Cemal, I. ve Atay, O., 1999b. Çine Çapari, Çine Tipi ve Menemen X Çine Tipi (F1) kuzularda kimi besi ve kesim özellikleri. Uluslararasi Hayvancilik'99 Kongresi, s.766-770, 21-24 Eylül 1999, Ege Üniversitesi Ziraat Fakültesi, Izmir.

Karaca, O., Yikilmaz, H., Cemal, I. ve Atay, O., 1999c. Çine Tipi, Menemen X Çine Tipi melezi (F1) ve Çine Çapari kuzularin kimi gelisme özellikleri. Uluslararasi Hayvancilik'99 Kongresi, s.771-776, 21-24 Eylül 1999, Ege Üniversitesi Ziraat Fakültesi, Izmir.

Karaca, O., Cemal, I., Altin, T., 2004. Yerli Çine Çapari Koyun Irkinin Genetik Kaynak Olarak Korunmasi Çalismalari. 4. Ulusal Zootekni Bilim Kongresi, 1-4 Eylül 2004 Süleyman Demirel Üniversitesi, Isparta

Karaca, O., Cemal, I., 2005. Gen kaynagi olarak risk altindaki yerli koyun irklarimizdan: Çine Çapari. HASAD Hayvancilik, 20, 239: 14-20.

Karaca, O., Cemal, I., Yilmaz, O., Yilmaz, M., 2009. Karya Koyunu. Türkiye Ulusal Koyunculuk Kongresi, 12-13 Subat, Izmir. s.225-234

Montaldo, H.H., Meza-Herrera, C.A., 1998. Use of molecular markers and major genes in the genetic imrovement of livestock. Elect J Biotech, 1, 83-89.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Pariset, L., Savarese, M.C., Cappuccio, I., Valentini, A., 2003. Use of microsatellites for genetic variation and inbreeding analysis in Sarda sheep flocks of central Italy. Italy Journal of Animal Breeding and Genetics, 120 (6) : 425-432.

Peakall, R., Smouse, P.E., 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes, 6, 288-295.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol, 24, 1596-1599.

Tapio, I., Tapio, M., Grislis, Z., Holm, L.E., Jeppsson, S., Kantanen, J., Miceikiene, I., Olsaker, I., Viinalass, H., Eythorsdottir, E., 2005. Unfolding of population structure in Baltic sheep breeds using microsatellite analysis. Heredity, 94, 448-456.

Tascon, D.C., Little John, R.P., Almeida, P.A.R., Crawford, A.M., 2000. Genetic variation within Merino Sheep breed: Analysis of closely related populationsusing microsatellites. Anim Genet, 31, 243-251, 2000.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X., 1997. POPGENE the user-friendlyshareware for population genetic analysis. University of Alberta, Canada, 1997. http://www.ualberta.ca/~fyeh/, Accessed: 05.05.2007

Handley, L.J.L., Byrne, K., Santucci, F., Townsend, S., Taylor, M., Bruford, M.W., Hewitt, G.M., 2007. Genetic structure of European sheep breeds. Heredity, 99, 620-631.

Pramod, S., Kumarasamy, P., Chandra, A.R.M., Sridevi, P., Rahumathulla, P.S., 2009. Molecular characterization of vembur sheep (Ovis aries) of South India based on microsatellites. Indian J Sci Technol, 2, 55-58.

Yilmaz, O., Karaca, O., 2012. Karya Koyunlarda Mikrosatellit Isaretleyicilerle Babalik Testi. Kafkas Üniversitesi Veteriner Fakültesi Dergisi,18 (5) :807-813. ISSN: 1309– 2251.