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Genetic Analyses of Some Central Anatolian Domestic Duck Populations with Inter Simple Sequence Repeat (ISSR): A Preliminary Study

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Abstract.- The aim of this study is to investigate the genetic structure of some domestic duck populations from Kirsehir and Yozgat provinces of the Central Anatolia region of Turkey. Blood samples were obtained from the *venae cutenea ulnaris* of 76 ducks from four different locations. Eleven ISSR primers produced 73 reproducible and bright bands. The number of polymorphic loci was 72 and the percentage of polymorphic loci was 98.6%. Gene diversity (H_T) in total population and magnitude of differentiation among populations (G_{ST}) were 0.198 and 0.183, respectively. The genetic distances between regions under investigation were found among 0.0157 and 0.0991. Analysis of molecular variance (AMOVA) reflected that 83% of within variation and 17% among population variation. First three given values of principle coordinate analysis explained 69.8% of total variation as 45.6, 13.8 and 10.4%, respectively. The dendrogram showed two main branches: one contains Kirsehir (L1, L2, L3), the other includes Yozgat region (L4). Shannon's Information index (*I*) value was 0. 331. The gene flow (*Nm)* among populations was analyzed and *Nm* value was estimated as 2.23 with the low level of differentiation among populations. The results indicated that genetic variations of some Central Anatolian domestic duck populations are determined using ISSR marker and might provide information for future breeding strategies.

Keywords: Duck population, genetic distance, ISSR primers.

INTRODUCTION

Ducks, one of the avian species, have economic, social and ecological value in far eastern countries, particularly in China, Vietnam, Malaysia, Indonesia etc. However; some European countries like France, Romania, Poland, and Ukraine are also rearing ducks (FAO, 2015). China is the leader of duck rearing (816,500,000) in the world. Goose and ducks meat including total poultry meat production of the world contain 7.2 %. Commercial production mostly involves liver production (Pingel, 2004).

In Turkey, the production level of domestic duck populations is approximately 357.000 according to FAO data (2015). However, the production levels of broiler and hen were very higher compared with the levels of other poultry production in Turkey (Table I). Unfortunately, there is no genetic information regarding domestic duck populations in Turkey. The possible explanation could be Avian Influenza. Because, during the years 2005-2008, a total of 2.5 million poultry were sacrificed and there was lack of information about how many ducks, goose or hens were sacrificed, respectively (www.tarim.gov.tr)

Table I.- Poultry and egg production, 2010-2012 (TurkStat, Turkey in Statistics, 2012).

Poultry and egg production (Thousand number)	2010	2011	2012
Duck	397	382	357
Turkey	2,942	2,563	2,761
Goose	716	680	676
Hen	234,918	237,873	253,712
Broiler	63,985	158,917	169,034
Laying hen	70,934	78,957	84,677
No. of hen eggs	11,840,396	12,954,686	14,910,774

Note. Egg production in villages is excluded starting from 2010.

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Authors' Contributions: RIT and MB performed the experiment work. AT collected blood samples and other statistical data. RIT designed the experiment and wrote the manuscript.

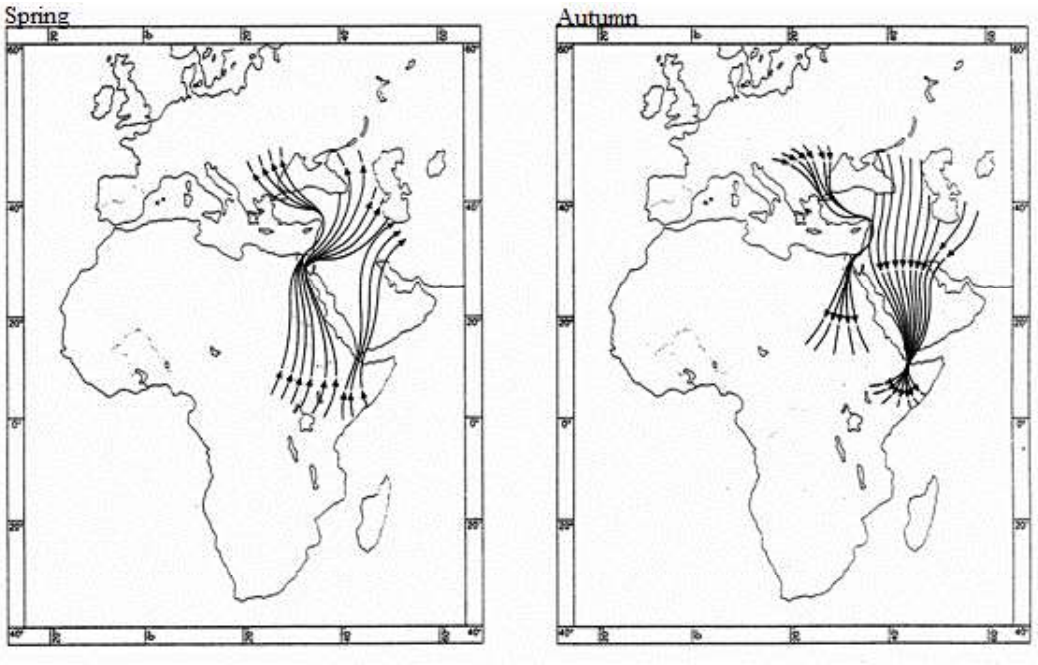

Fig. 1. Migration routes for waterfowl in Turkey in spring and autumn periods. (http://kaced.org/trbiyolojikcesitlilik.php)

Table II.- The number of scarified poultry and affected provinces in Turkey.

Years	The provinces affected by the disease	The number of sacrificed poultry
2005-2006	54	2.500000
2007	2	27.190
2008	5	7.477

(Table II). In the Western European countries such as Germany and France, it has been reported that the number of animals sacrificed poultry has reached to 10 million (Sarnic, 2006).

Rather than genetic studies especially in duck populations in Turkey, several investigators generally prefer to study on breeding or feeding studies (Sari *et al*., 2013; Demir *et al*., 2010; Erisir *et al*., 2009; Alpay, 2008; Arslan *et al*., 2003; Isguzar, 2006; Isguzar and Testik, 1999). In recent times, many studies have been documented about distributions and breeding regions of waterfowls especially white headed duck in Turkey (Nergiz *et al.,* 2014; Perktas *et al.*, 2006; Mun˜oz-Fuentes *et al*., 2005; Kirwan, 1994; Green *et al*., 1996). Hence, Turkey is a key point for waterfowls from their migration routes (Fig. 1). During spring and autumn period millions of waterfowl located in wetlands in Turkey. Also endangered species like white headed duck locate in these regions during their migration period (Li and Mundkur, 2003; Mun˜oz-Fuentes *et al*., 2005).

In the world, knowledge of genetic variation of duck was obtained by different molecular methods such as Random Amplified Polymorphic DNA (Su *et al*., 2006), microsatellite (Hui-Fang *et al*., 2010; Su and Chen, 2009; Su *et al*., 2007) and mitochondrial DNA (Mun˜oz-Fuentes *et al*., 2005) whereas there are no documentations for duck populations using Inter-simple sequence repeat (ISSR). The ISSR based on PCR technique is implemented to determine variation from the regions between microsatellites. ISSR is a fast, efficient and inexpensive is a molecular technique and it is widely used in the determination genetic relatedness among populations in many organisms (Zietkiewicz *et al*., 1994). It is no need to use of radioactivity. It is reliable and includes more

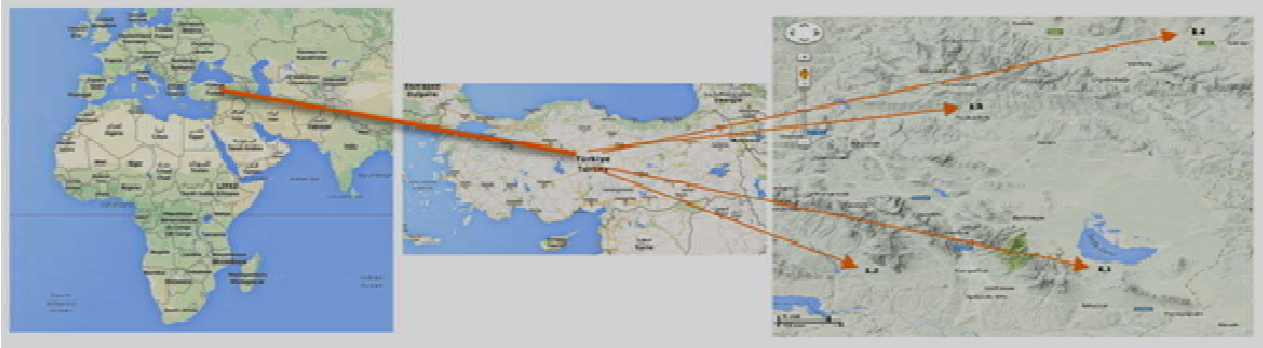

Fig. 2. Samples collected regions (Dulkadirli (39º50'N-34º14'E), Seyfe Lake region (39º14'N-34º45'E), Hirfanlı Dam region (39º73'N-34º63'E) in Kirsehir and Saray (39º19'N-33º63'E) in Yozgat).

information than other dominant molecular techniques using single arbitrary primers (Gradzielewska, 2011). ISSR have also been used for gene mapping, population genetic studies, identification of breed of species (Bornet and Branchard, 2001).

In literature, knowledge on the identification of genetic variations using ISSR in the Central Anatolian duck populations has not yet been documented. Herewith, the aim of this study is to investigate the genetic structure of some domestic duck populations in Kirsehir and Yozgat provinces of the Central Anatolia in Turkey. This study is the first report including molecular analysis for determination of genetic diversity of domestic duck population in Turkey.

MATERIALS AND METHODS

Blood samples were obtained from the *venae cutenea ulnaris* of 76 ducks from four different locations in Central Anatolia (Dulkadirli (39º50'N-34º14'E), Seyfe Lake region (39º14'N-34º45'E), Hirfanlı Dam region (39º73'N-34º63'E) in Kirsehir and Saray (39º19'N-33º63'E) in Yozgat) (Fig. 2). Eleven primers were given in Table III. Genomic DNAs were isolated from blood samples as described by Dunnington *et al*. (1990). PCR mixture were composed of 200um dNTPs, 0,2um primer, 20ng DNA, 2U taq DNA polymerase, 1X Taq Buffer in 25 µl total reaction volume. Amplifications were performed as followed programs 3 min at 94°C (1 Cycle), followed by 1 min 94°C, 45 sec 50-56°C, and 2 min 72°C (35 cycle), followed by final extension at 72°C for 5 min. Amplification products were resolved by electrophoresis in 1.2% agarose gel with 0.8 X TBE buffer. After electrophoresis, gels were stained with ethidium bromide solution (10 m/l ml) and visualized under UV (Fig. 3).

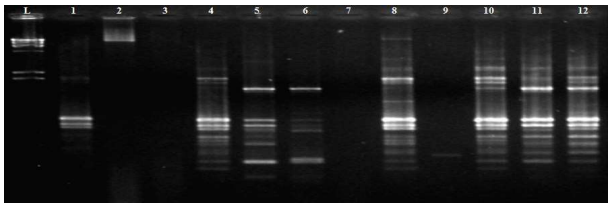

Fig. 3. PCR results for $(AC)_8GA$ ISSR primers (Line 1: DNA Ladder; Line 2-13: DNA Samples).

Table III.- Primer sequences, annealing temperatures and number of bands per primer.

Primers	Annealing temperature (ºC)	# of bands per primer
$(CA)_8G$	50	6
$(AG)_8G$	55	5
$(AC)_8GA$	54	11
$(AC)_8CA$	54	4
$(GA)_8GG$	56	5
$(GGGGT)_3G$	55	5
$(GA)_8CC$	54	6
$(TG)_8A$	54	6
$(CT)_8GC$	54	10
$(CAG)_5$	54	9
$(CA)_8AGC$	55	8

Polymorphic and monomorphic bands were scored for ISSR. Percent of polymorphic bands,

gene diversity (Nei, 1973), Nei's (1978) genetic distance and genetic identity were estimated in populations. Shannon's information index (*I)* (Lewontin, 1972) is used for measuring species diversity in ecology studies whereas it also estimates genetic variability in genetic studies. Therefore, genetic variability of this study was estimated using Shannon's information index. The coefficients of gene differentiation (G_{ST}), and gene flow *(Nm)* were calculated using POPGENE 1.31 software (Yeh *et al*., 1999). Analysis of Molecular Variance (AMOVA) and Principle Coordinate Analyses (PCA) were done using Genalex6 software program (Peakall and Smouse, 2006). UPGMA tree was performed based on Nei's (1978) genetic distance using NTSYSpc V2.20e (Rohlf, 2000).

RESULTS

Eleven ISSR primers produced 73 reproducible and bright bands. All bands were polymorphic when all populations were evaluated. The number of polymorphic loci was 72 and the percentage of polymorphic loci was 98.63%. The effective number of alleles (Ne) was estimated as 1.26. Gene diversity (H_T) according to Nei's (1973) and Shannon's information index (*I*) values were calculated as 0.198 and 0.331, respectively. Mean diversity within subpopulation (H_S), total genetic diversity in the all populations (H_T), and magnitude of differentiation among populations (G_{ST}) values were given in Table IV. G_{ST} value was 0.183 and this value indicated that there was relatively low level of genetic differentiation among populations. The gene flow among populations was analyzed and *Nm* value was estimated as 2.23 and the present result showed that low level of differentiations were found among populations.

Analysis of Molecular Variance (AMOVA) respected that the total genetic variation includes 83% within, 17% among populations. First three Eigen values of PCA explained 69.8% of total variation. First, second and three Eigen values were 45.6, 13.8 and 10.4%, respectively. According to Nei's (1978) genetic distance, L3 (samples collected from Dulkadirli in Kirsehir) and L4 (samples collected from Saray in Yozgat) were the most distant (0.0991), also L1 (Seyfe lake region) and L2 (close to Hirfanli Dam) were the least distant populations (0.0157) (Table V). Cluster analysis revealed two main branches, one leading to duck populations in Kirsehir, the other one including Yozgat region (Fig. 4).

Table IV.- Total genetic diversity in the all populations (H_T), mean diversity within subpopulation (H_S), coefficient of gene differentiation (G_{ST}), Gene flow (Nm) and Shannon's information index (I) values.

	Mean	St. Dev
N	76	
H_T	0.198	0.0206
H_S	0.156	0.0122
G_{ST}	0.183	
Nm*	2.230	
I*	0.331	0.1550

* I (Lewontin 1972); Nm = estimate of gene flow from Gst (McDermott and McDonald, 1993).

Table V.- Genetic identity (above diagonal) and distance (below diagonal) values for studied populations (L1: Seyfe Lake region,L2: Hirfanlı Dam region, L3: Dulkadirli in Kirsehir and L4: Saray in Yozgat).

	L1	L2	L3	L4
L1	****	0.9844	0.9513	0.9654
L2	0.0157	****	0.9822	0.9462
L3	0.0500	0.0180	****	0.9057
L4	0.0353	0.0553	0.0991	****

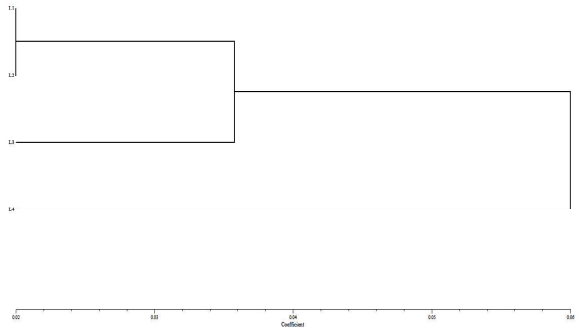

Fig. 4. Dendrogram Based Nei's (1978) Genetic distance using UPGMA method (L1: Seyfe Lake region,L2: Hirfanlı Dam region, L3: Dulkadirli in Kirsehir and L4: Saray in Yozgat).

DISCUSSION

Genetic diversities were determined by many studies in both wild and domestic duck populations for many countries such as China, Spain and USA etc. These studies were conducted on duck populations using Microsatellite, mtDNA, RAPD and AFLP markers (Munoz- Fuentes *et al*., 2006; Su *et al*., 2007; Su and Chen, 2009). In Turkey, the genetic information is still scarce about domestic duck populations, whereas the previous studies about domestic duck populations mostly contain feeding, management, and performance in Turkey. In these previous studies, cost benefit analyses of various housing systems, performance, carcass characteristics and different feeding systems were studied by some earlier investigators in different publications (Demir *et al*., 2010; Erisir *et al.*, 2009; Isguzar, 2006).

Very little genetic information is known about the wild duck populations existing for Turkey. The study regarding the wild duck populations was carried out on endangered white-headed duck population (*Oxyura leucocephala*) in which genetic structure and genetic diversity were determined by using mtDNA for many countries including also Turkey (Munoz- Fuentes *et al*., 2005). Two different haplotypes were genetically identified using mtDNA methods for wild white-headed duck populations for samples taken from Turkey (Munoz-Fuentes *et al.*, 2005). The distribution and breeding status of wild duck populations and other waterfowl species were generally determined for many lakes and marshes of Turkey (Nergiz *et al*., 2014; Biricik and Karakas, 2011; Perktas *et al*., 2006 Green *et al*., 1996; Kirwan, 1994). One of these studies was performed in Seyfe Lake located in Kirsehir and the populations of waterfowls were informed such as shelduck, rudy shelduck and etc (Perktas *et al.,* 2006). This location overlaps in the present study and diversity of waterfowl species were informed in Seyfe Lake region.

The data represented here reveals that there is low level differentiation among the studied populations, and the present study is the first document for the determination of genetic inventory for domestic duck populations. In conclusion, genetic studies about wild and domestic duck populations were relatively limited in Turkey. More studies are still needed to determine genetic inventory about duck populations both wild and domestic which are very important for gene sources of Turkey. The information about genetic relationships and detection of genetic inventory for duck populations has several important applications for its genetic improvement and breeding in Turkey for development of future breeding strategies.

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Statement about conflict of interest

There is no conflict of interest among authors.

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