



A comparison of genetic variations among native and some local chicken populations in Turkey

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Received: 02-02-2016

Accepted: 07-04-2016

DOI:10.18805/ijar.10278

ABSTRACT

Genetic variations among native and local chicken populations in six different locations across Turkey were established using 15 ISSR primers, and produced 87 bright and reproducible bands. According to pairwise genetic differentiation among the populations (G_{ST}), the highest genetic differentiation was determined between the Samsun and Yozgat population and the lowest was observed between the Dulkadirli and Budak populations. Shannon's index was calculated to be 0.239. The gene flow (N_m) among the populations was estimated to be 3.489. Analysis of Molecular Variance (AMOVA) identified 13% of the total genetic variation between the populations and the rest of the differences were 87% within the populations. Cluster analysis revealed two main branches, one leading to the domestic chicken population collected from Samsun in the Black Sea region of Turkey, the other branch clustered into two branches; one branch consisting of the Denizli Native Chicken population and the other one of domestic chicken populations sampled from Central Anatolia populations. The study is important to clarify the indigenous chicken genetic resources in Turkey. The results could be used for future breeding research conducted by either the public or private sectors.

Key words: Genetic variation, Native and domestic chickens, ISSR.

INTRODUCTION

The domestic chicken originates from South-East Asia and is widespread throughout the world. For a long time, based on morphology and protein alone, their wild ancestors were considered to be the red jungle fowl (*G.gallus*) (Kanginakudru *et al.*, 2008; Tixier-Boichard *et al.*, 2011). As a result of human migration along ancient trade routes, by the Iron Age the chicken had already spread to Africa and Europe (Lyimo *et al.*, 2014). Two routes in particular, northern (from China to Russia) and southern (from Persia to Greek), played important roles in the increase of chickens throughout Europe (West and Zhou 1988; Crawford, 1990; 1995; Tixier-Boichard *et al.* 2011; Flinket *al.* 2014; Lyimo *et al.*, 2014).

Over the years, chickens have been used for food, human cultural activities, such as religious ceremonies, and decoration (Kaya and Yildiz, 2014). Whatever they were used for, as a consequence of chicken breeding activities, modern chickens were distributed to many regions of the world. As a result of cross-breeding, either by humans or naturally, over time native breeds can now be found in many countries (Kaya and Yildiz, 2014). Day by day, the genetic diversity of local chicken populations is being lost, mainly as the result of intensive selective breeding programs (Granevitze *et al.*, 2007). The development of valuable genotypes and desirable

traits in chicken populations may have resulted in the substitution of local chicken populations by commercial populations, and this situation may lead to a decrease in genetic diversity (Pisenti *et al.* 2001; Granevitze *et al.*, 2007). The recognition of local or native chicken populations and conserving them as founder genetic sources is critical for future breeding strategies and management (Kaya and Yildiz, 2014).

Since ancient times, Anatolia has been the keystone of many civilizations; hence, it is hard to predict when chicken gene pools were originally formed in Anatolia (Kaya and Yildiz, 2014). Gerze, Denizli, and Sultan are well known native chicken breeds in Turkey. The determination of genetic variations was specifically conducted on Denizli and Gerze native populations using molecular techniques (Kaya and Yildiz, 2008; Taskesen, 2010; Mercan and Okumus, 2015). Using different molecular markers, some genetic studies have been conducted on both native and commercial chicken breeds in Turkey and microsatellite variations, Mitochondrial DNA (mtDNA) and Random Amplified Polymorphic DNA (RAPD) methods have been frequently used to determine the genetic variations of chicken populations in Turkey (Ivgin and Bilgen, 2002; Okumus and Kaya, 2005; Kirdag, 2007; Kaya and Yildiz, 2008; Taskesen, 2010; Mercan and Okumus, 2015).

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In this current study, Inter Simple Sequence Repeat (ISSR) was used to determine genetic variations among native and domestic chicken populations. ISSR, as a molecular marker, does not need radioactivity and it is simple, quick, and inexpensive; it also shows high polymorphism. The ISSR uses primers that are complimentary to a single SSR (Zietkiewicz *et al.* 1994). It has been widely used for determination of genetic relationship among populations and gene mapping of many organisms (Bornet and Branchard, 2004; Ye *et al.*, 2005).

In the mapping of chicken genome, different methods such as chromosome scraping, flow cytometry, the construction of chromosome-specific libraries, genetic analysis based upon polymorphic DNA markers, and *in situ* hybridization have all been used. But sometimes analyzes based on polymorphic DNA markers (RAPD, RFLP, VNTR, SSR, and CR1-PCR) have not been associated with each other (Sazanov *et al.*, 1996). Research on commercial lines, such as studies frequently carried out with White Leghorn chicken breeds were performed using mtDNA, RAPD and SSR in order to reveal genetic similarities among populations (Nahashon *et al.*, 2010). The native breeds, Denizli and Gerze in Turkey, well adapted to extreme environmental conditions in Aegean and west part of Central Anatolia region, have resistant to many diseases and have survived up to now (Kaplan and Aksoy, 2009). In research on Denizli native breeds there has been shown to have a significant positive correlation between body and egg weight and also between egg production and egg weight. It was discovered that positive selection can be made using egg production, egg and body weight characteristics and it also showed that native breeds can be used for various selection programs in native breeds (Atasoy and Gurcan, 2010).

The objectives of the study conducted using ISSR method was to compare and determine genetic relationships among Kirsehir and Yozgat domestic chicken breeds, which

were located in Central Anatolia, Samsun (Black sea region) and Denizli native breed, in Turkey.

MATERIALS AND METHODS

Sampling: Blood samples were collected from the Venae cutenea ulnaris of 132 chickens in six different locations in Turkey: Dulkadirli (39°30'43.14"N-34°9'47.09"E), Budak (39°8'24.26"N-34°27'13.93"E), Kaman (39°21'32.18"N-33°43'24.18"E) in Kirsehir, Yozgat 39°41'34.9"N 34°39'11.7"E, Samsun (41°11'27.38"N-36°43'33.07"E) and Denizli (37°46'59.61"N-29°5'48.47"E) (Fig 1.). In local populations, blood samples were collected from chickens, which breeders had bred using their own flock for very long time, and who did not obtain eggs for hatching from other commercial farms. A total of 112 samples over five regions were used with these features. Twenty blood samples were collected from the Denizli native breed, which were already characterized and found to be a native breed by the Denizli Directorate of Provincial Food, Agriculture and Livestock. Fifteen ISSR primers (Khatab, 2011; Bornet and Branchard, 2004; ISSR primer set from University of British Columbia) were used as given in Table 1.

Molecular Analyses : Genomic DNAs were isolated from blood samples, using commercial DNA isolation kits (Fermentas, K512). The PCR mixtures were composed of 25ng DNA, 200um dNTPs, 0.2um primer, 1.5U Taq DNA polymerase, 1X Taq buffer (Fermentase) in 25 ul total reaction volume (Khatab, 2011). PCR amplification was done in a Thermal Cycler (Clever Scientific, GTC96S). PCR was performed for two minutes at 94 °C (1 cycle), followed by one minute at 94 °C, 45 sec 50-56 °C, two minutes at 72 °C (30 cycles), and a final extension at 72 °C for five minutes. The amplification products were resolved by electrophoresis in 1.2% agarose gel (Sigma) with 1 X TBE buffer. Following electrophoresis, the gels were stained with ethidium bromide solution and visualized under UV.

Table 1: Primer sequences, annealing temperatures and number of bands per primer

Primer Sequences	(References)	Annealing Temperature (°C)	# of bands per primer
(AG)8G	(UBC-809)	54	4
(GA)8T	(UBC-810)	50	6
(CA)8G	(UBC-818)	55	7
(TG)8A	(UBC-828)	55	4
(GA)8YC	(UBC-841)	54	5
(GA)8YG	(UBC-842)	54	7
(CT) ₈ RC	(UBC-844)	54	6
(CA)8RG	(UBC-848)	56	9
(AC)8YA	(UBC-856)	54	10
(AC)8GA	(Khatab, 2011)	54	8
(CT)8TT	(Khatab, 2011)	56	4
(AC)8CA	(Khatab, 2011)	55	4
(GATA)2(GACA)2	(Khatab, 2011)	54	5
(GGGGT)3G	(Khatab, 2011)	54	4
(CAG)5	(Bornet & Branchard, 2004)	54	4

(R=G or A; Y=C or T)



Fig 1: Collected samples from the six different locations

Statistical analyses: The bands, polymorphic and monomorphic for the ISSR marker, were scored as zero and one. The gene diversity of total (H_T) and within-population (H_S) were calculated to estimate the genetic variation according to Nei (1973). Effective allele numbers (N_e) were estimated according to Kimura and Crow (1964). The coefficients of the gene differentiation (G_{ST}), gene flow (Nm) and Shannon's information index (I) (Lewontin, 1972) were estimated using POPGENE 1.31 software (Yeh *et al.*, 1999). The UPGMA tree was conducted, based on Nei's (1978) genetic distance, using NTSYSpc V2.20e (Rohlf, 2000). Analysis of Molecular Variance (AMOVA) and Principle Coordinate Analyses (PCoA) were made using the Genalex6 software program (Peakall and Smouse, 2006).

RESULTS AND DISCUSSION

A total of 87 reproducible and bright ISSR bands were produced with 15 primers (Table I). All the bands were polymorphic in all the populations. The mean number of effective alleles (N_e) was 0.157 in all populations. Gene diversity (H_T) for total populations and mean diversity within subpopulation (H_S) according to Nei's (1973) was estimated to be 0.135 and 0.118, respectively. Shannon's information index (I) estimates genetic variability in genetic studies and it also measures species diversity in ecology studies. In this current study, Shannon's index was calculated to be 0.239. The gene flow (Nm) among the populations was estimated to be 3.489 and the result showed that a low level of divergences was determined between the populations.

Magnitude of differentiation among the populations (G_{ST}) value was 0.125 and this value indicated that there was a moderate level of genetic differentiation among the

populations. Pairwise genetic differentiations among the population (G_{ST}) values are given in Table 2. The highest genetic differentiation was established between the Samsun and Yozgat populations and the lowest was observed to be between the Dulkadirli and Budak in Kirsehir populations.

Analysis of Molecular Variance (AMOVA) distinguished 13% of total genetic variation between populations and the rest of the differences were 87% within the populations. The first three Eigen values of PCoA explained 60.1% of total variation. The first, second and three Eigen values were 42.8, 9.1 and 8.2%, respectively. Genetic distance is presented (Nei, 1978) and the highest genetic distance was detected to be 0.436 between the Samsun and Denizli populations (Table 2). The lowest values were observed between the Yozgat and Kaman in Kirsehir populations (0.0021).

Cluster analysis revealed two main branches, one leading to the domestic chicken populations collected from Samsun located in Black Sea region in Turkey. The other branch clustered into two subdivisions; one consisted of the Denizli Native Chicken population and the other one of the domestic chicken populations sampled from the Central Anatolia populations (Dulkadirli, Budak, Kaman in Kirsehir and Yozgat) (Fig. 2).

Up to now, throughout the world, genetic variations and the original verification of chicken populations have been conducted by many studies using different molecular markers. These studies contain both commercial and native chicken populations and use markers such as mtDNA, AFLP, RAPD, Microsatellite and SNP (Sharma *et al.*, 2001; International Chicken Polymorphism Map Consortium,

Table 2: Magnitude of genetic differentiation among pairwise population (G_{ST}) (below diagonal) and Nei's (1978) genetic distance (above diagonal) values.

	Dulkadirli	Budak	Kaman	Yozgat	Samsun	Denizli
Dulkadirli	***	0.0033	0.0067	0.009	0.0387	0.094
Budak	0.0222	***	0.0065	0.0083	0.0411	0.0063
Kaman	0.0377	0.0371	***	0.0021	0.0484	0.0091
Yozgat	0.0504	0.0485	0.0236	***	0.0555	0.007
Samsun	0.1026	0.1158	0.1347	0.1591	***	0.436
Denizli	0.0479	0.0366	0.052	0.0472	0.1261	***

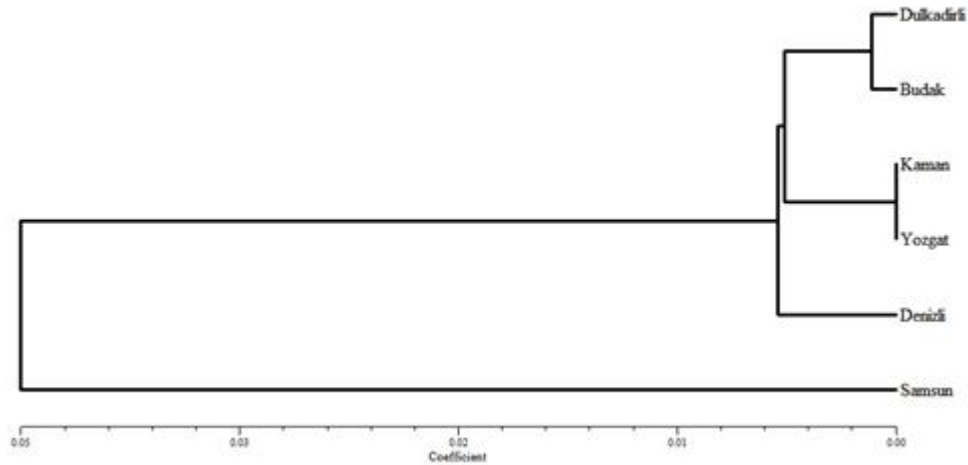


Fig 2: Dendrogram Based Nei's (1978) Genetic distance using UPGMA method

2004; Shahbazi *et al.*, 2007; Gongora *et al.*, 2009; Riztyan *et al.*, 2011; Storey *et al.* 2012).

In Turkey, several studies have revealed the genetic information of chicken populations. Prior to the 2000s, in order to detect genetic variation in Turkey, researchers studied allozyme and protein polymorphism. These studies gave us only very limited knowledge concerning genetic variations. (Bilgen *et al.*, 1999; Aksoy *et al.*, 2000). Since the 2000s, in order to determine genetic variation, some molecular studies based on DNA have been conducted in Turkey (Ivgin and Bilgen, 2002; Okumus and Kaya, 2005; Kirdag, 2007; Kaya and Yildiz, 2008; Taskesen, 2010; Mercan and Okumus, 2015). Initially, genetic distance and similarities were detected among meat and layer pure line chickens in Turkey, using the RAPD technique (Ivgin and Bilgen, 2002; Okumus and Kaya, 2005); MtDNA variations were also detected in native and commercial populations. In one of these studies, 12S, 16S, D-loop and CytB gene region variations were performed in the Denizli, Gerze native genotype and Brahma breeds. Ten different nucleotides were determined between Brahma and Denizli and 14 different nucleotides were observed between Brahma and Gerze (Kirdag, 2007). Another study was performed using the mtDNA marker, in order to determine the genetic relationship between the Denizli native breed and Red Jungle Fowl (RJF) subspecies, Shamo, Silky, Laos, Plymouth, New Hampshire Red, two different White Leghorn, and Gerze native chicken breeds (Taskesen, 2010). In that particular study, two different haplotypes were discovered for Denizli native chickens (Taskesen, 2010). Mitochondrial DNA D-loop and 12S regions were studied in Denizli native chicken and the result of the study gave informative data for local breed in

Turkey (Karaman and Kirdag, 2012). The microsatellite genetic variations of two native breeds, Denizli and Gerze, were determined to be ten microsatellites (Kaya and Yildiz, 2008). The genetic structures of local chicken populations of the Central Black Sea region and two commercial chicken populations in Turkey, were determined and compared using twenty eight microsatellite loci (Mercan and Okumus 2015). Their study identified that local chicken populations had a higher genetic diversity than commercial hybrid populations (Mercan and Okumus, 2015).

So far, the studies have shown that Denizli and Gerze are genetically different native breeds in Turkey. Our study showed that the Central Anatolia chicken populations are clustered together and close to the Denizli native breeds. On the other hand, results have illustrated that the Samsun samples, collected from mountain villages in the Black Sea region, have varied from the populations in Denizli and other regions. The samples collected from the Black Sea region should be genetically analyzed with intensive sampling and compared with the Gerze breed of native chicken.

The study showed that there were the indigenous chicken genetic resources in Turkey. The public or private sectors could be used in the present results in order to improve future breeding strategies for chicken populations.

ACKNOWLEDGEMENT

We are grateful to Denizli, Yozgat, Samsun and Kirsehir Directorate of Provincial Food, Agriculture and Livestock for their help. All samples were collected by Veterinarians who worked in the Directorates and animals were not given any damaged during procedure. There is no conflict of interest among the authors.

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