Genetic differentiation among three populations of Iranian guardian dogs

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Abstract

Several breeds of dogs can be found in Iran. Some of these breeds are completely different in their phenotype while others are similar. These animals are kept in rural families as guard animals for their herds. One of the indicators of genetic diversity within and between populations is *Y* sex chromosome haplotype which is inherited from sires to their male offspring. In order to study the genetic diversity of a part of *Y* sex chromosome between and within the populations of *Sarabi*, *Sangsari* and *Afshari* dogs, total number of 21 non inbred male dogs were sampled from these populations. Blood samples collected from these animals were transferred into laboratory by EDTA containers. DNA extraction was applied using extraction kits and forward and reverse primers were designed using Oligo5 software. After sequencing, results were analyzed by Finch and Mega4 softwares. Analyzing the replicated sequences showed that all of the sequences were conserved and having repeated sequences caused the existence of slip pages. In this study, both neighbor joint approach and unweighted pair-group method of the arithmetic average (UPGMA) were utilized to draw phylogenic tree. Results showed that these three populations of dogs, based on their genetics, are completely different from each other which is very important in conservation of pure breeds and avoiding crossbreeding between distinct populations.

Keywords: Iranian dogs, genetic differentiation, Y chromosome

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Introduction

In most countries of the world dogs are used as police and guardian animals after they are well trained and bred. In some countries like New Zealand and Australia dogs are also used as herd animals in ranges and pastures. In Iran different breeds of dogs exist which some of them are completely different from each other while others may show some resemblance and kept in rural herds. Unfortunately to date there has not been a complete and comprehensive study about genetic similarities and differences among these breeds. Different names of some breeds may be only due to different geographical regions where they are bred without having any genetic differences or vise versa. Therefore according to adaptation of these breeds with environmental conditions of Iran during a period of time, it is necessary to have a vast survey about their characteristics and determination of genetic differences among them. Since basic principles of animal breeding have not been

implemented for these animals and also because of unorganized mating, the probability of hybridization has increased. It is also possible to study the genetic variation and phylogenic relations of these animals using modern molecular methods. Caryotype evaluation and genome wide studies of Y sex chromosome which is inherited from sires to offspring and determination of genetic similarity and differentiation among three populations of Iranian guardian dogs is one of the possible activities in this field.

There have been few studies about characterization of dog populations, assessment of genetic distances and categorizing of breeds. Results of a haplotype analysis of Y chromosome in purebred animals showed that in 824 individuals, only 67 haplotypes were found which was due to the existence of common haplotypes among different breeds and low diversity of haplotypes inside the species and only 15 breeds could be said to have exclusive haplotypes. Also for 26 breeds of total 50 breeds exclusive haplotypes were found (3). In another study, Y chromosome sequencing showed exclusive haplotypes in 10 breeds of Asian, European and American dogs while in Africa only nine haplotypes were found. Genetic distance between haplotypes revealed that the origin of such animals maybe at least five haplotypes of wolves (4). Haplotyping of Y chromosome in Scandinavian wolves showed that in sampled animals there are 17 different haplotypes (7). Mitochondrial DNA sequencing in 654 dogs of main breeds of whole world showed that wolves are maternal ancestors of modern dogs. All the sequences belonged to three global phylogenic groups and this result indicates that dogs belong to one genetic pool. One study revealed that genetic diversity in East Asian dogs is higher in comparison to other parts of the world. Phylogenic pattern shows that Eastern Asia is the origin of domesticated dogs (around 15000 years ago) (8).

Combination use of parental genetic markers for identification of dog-wolf hybrids showed that the underlying sequences of Y chromosome in these hybrid individuals can not bee found in Scandinavian species of wolves, even though their mitochondrial DNA is correlated with ones of wolves (8).

Location and characteristics of nucleotide sequences of Y chromosome DNA in Canidae family indicates that a specific sequence of 658 bp is located in end part of pseudo autosomal strand while the sequence of hybrid SRY gene is located close to centromere (5).

In this study our aim was the determination of genetic diversity in a part of Y sex chromosome, construction of genetic distance matrix and finally drawing the phylogenic tree for three populations of Iranian guardian dogs.

Material and Methods

In this research blood samples of 21 non-inbred male dogs from three populations of Sarabi, Sangsari and Afshari were collected. Blood samples were transferred to laboratory in tubes containing EDTA by cold chain conditions. DNA extraction was performed using Gene-Pajoohan-Pooya ready kits which are based on salt detergent method and Chloroform. In order to evaluate quantity and quality of DNA, NanoDrop instrument was used. Primers which were designed using Oligo5 software and their exclusivity were check in USCC and NCBI websites. Primer synthesis was done in Germany by Metabion co. primers are shown in table 1.

Table 1. Properties of primers

5′ -TGCCTGTCTTTGTAATCCCC- 3′	CaY1-F
5′ -CTCCCACCTCCTATCCCAC- 3′	CaY1-R
5′ -TCAAATGATAAAATGGTCCAG- 3′	CaY2-F
5′ -AACATCCCACATTTCTCAGAC- 3′	CaY2-R

For optimizing the reactions, the first parameter on concern was the temperature. Next step in optimizing process is to eradicate the nonexclusive bands and obtaining sharp bands which was done by using magnesium ion in PCR reactions. The optimization process is shown in table 2.

Table 2. Optimum quantities in polymerase chain reaction (micro liter)

dNTP	DNA	MgCl2	Taq polymerase	Reverse primer	Forward primer	Buffer	Aqua
0.4	3	1.2	0.2	2	2	2	9.2

Total volume of this reaction reached to 20 micro liters. 250 units of Taq polymerase enzyme and 25 millimolar $MgCl_2$ were used. PCR steps are shown in table 3.

Table 3. PCR steps

Temperature C^o	**Time**	**PCR steps**
95	5 min.	Initiation
95	30 sec.	Denaturation
58	50 sec.	Annealing
72	50 sec.	Extension
72	10 min.	Final Extension

Steps 2, 3 and 4 were replicated 35 times. The PCR products then were transferred to %1 Agarose gel for monitoring the bands formed. A 100 bp ruler was also loaded. The Agarose gel was transferred to Geldoc and the PCR bands were evaluated, photographed and then 20 micro liter of this product nevig saw to laboratory for further sequencings. Final sequences were analyzed using MEGA4 and FinchTV softwares.

Results

Figure 1 shows one sample of the bands obtained.

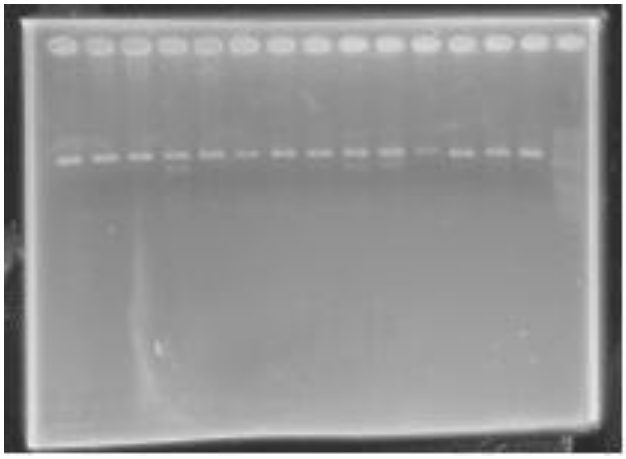

Figure 1. One sample of the bands photographed

Phylogenic tree which is also called evolutionary tree shows the evolutionary relations among different groups of living individuals which have common ancestors. There are several methods of drawing this tree which in this research two of those methods were applied.

a. Neighbor Joint method

The base of this method is to minimize the length of phylogenic tree. Optimized tree for our data is shown in figure 2. This tree has been rescaled and the length of its branches is according to evolutionary distances. Phylogenic distances are calculated by Maximum Likelihood Composite Method and are described by number of base substitution in each location. All locations which had lost data were omitted from analysis. There were total of 137 locations in final dataset.

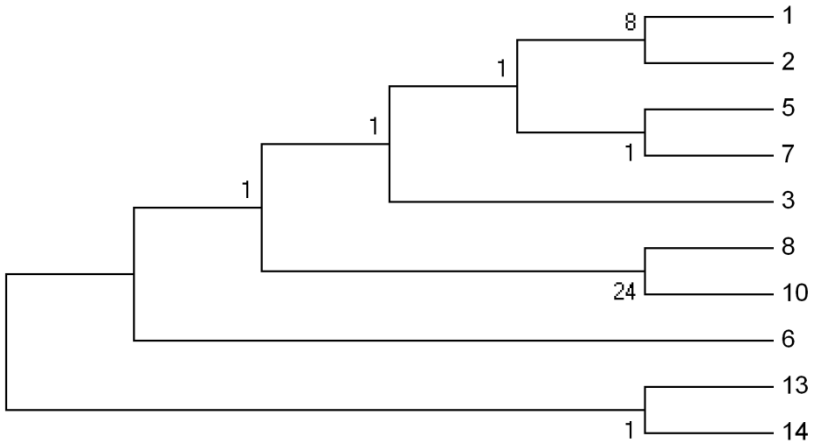

Fig 2. Phylogenic tree using Neighbor Joint Method

Distances less than 25 units show differences in phylogeny. The result of this tree revels the dissimilarity between all three populations.

b. Unweighted Pair Group Method with Arithmetic Mean

This method is one the simplest ways of drawing phylogenic tree. Phylogenic tree for our data is shown in figure 4 which shows the differences between all three populations.

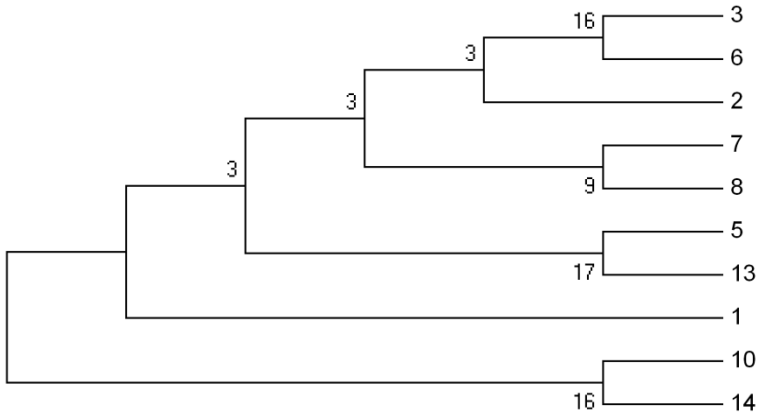

Fig 3. Phylogenic tree drawn using UPGMA method

Another test which was used here is the Test of the homogeneity of Substitution Patterns between Sequences. This test uses Monte Carlo simulation with 1000 replicates and estimates the probabilities which are shown in table below. P probabilities less than 0.05 are known as significant values. Results here show that there is not any similarity between our populations.

1	2	3	4	5	6	7	8	9	10]
	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]
1.000		[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]
1.000	1.000		[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]
1.000	1.000	1.000		[0.000]	[0.000]	[0.000]	[0.000]	[0.000]	[0.000]
1.000	1.000	1.000	1.000		[0.000]	[0.000]	[0.000]	[0.000]	[0.000]
1.000	1.000	1.000	1.000	1.000		[0.000]	[0.000]	[0.000]	[0.000]
1.000	1.000	1.000	1.000	1.000	1.000		[0.000]	[0.000]	[0.000]
1.000	1.000	1.000	1.000	1.000	1.000	1.000		[0.000]	[0.000]
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		[0.000]
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

Fig 4. Matrix of homogeneity of Substitution Patterns between Sequences

Replicated sequences were analyzed by FinchTV software. Results show that all the sequences are conservative. Repeated (For example poly T from segment 170 to segment 189) caused the existence of slip sheets which made some difficulties in sequencing of next segments. This situation is shown in figure 6. Since each individual has only one Y chromosome the probability of frame shift phenomenon decreases here. Here the overlapping of graphs maybe a results of mistakes in sequencing.

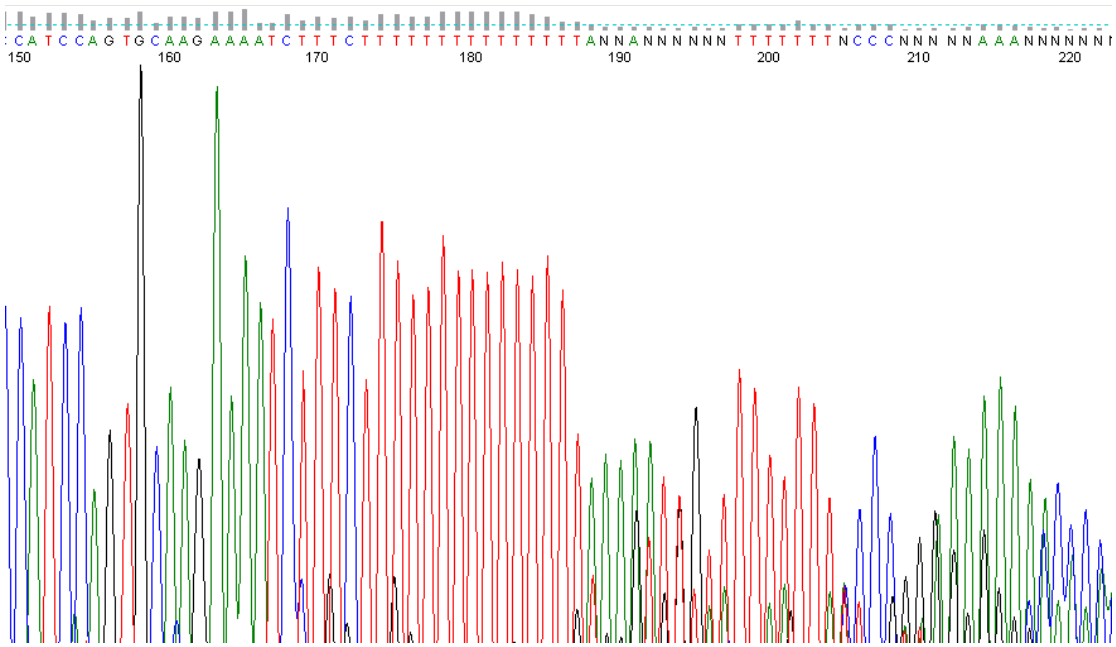

Fig 5. The result of sequencing

Discussion

Is this study the existence of repeated sequences created some slip sheets which made some difficulties in sequencing of next segments and some mistakes in reading of alleles. During replication process DNA polymerase enzyme may slip and consequently some products with different sizes may be made which are different from one to five units from original products.

The results of phylogenic tree from both NJ and UPGMA methods indicate differences between all three populations. The assessment of genetic distance between populations is an estimate of differentiation time. Low estimates of genetic distance may refer to the complete differentiation of populations but there may be a short time passed from this separation. Two mechanisms of genetic mutation and drift cause differences in allelic frequencies of null loci. The differences between these three populations make it necessary to study each population separately. In this regard breeding strategies in each region according to the requirements of each population must be designated and breeding characteristics must be defined independently.

There are some similarities between two methods of drawing the phylogenic tree which leads to correlation of these two criteria. Bannasch et al. (2005) used haplotype analysis of Y chromosome in purebred dogs and found that in all 824 dogs, only 67 haplotypes were recognized and the main reason was said to be the common haplotypes among dog breeds and low diversity in each species. 15 breeds were recognized according to their exclusive haplotypes.

After realizing the genetic differences between populations of interest, proper policies may be settled to save the pure breed resources and improving their genetic makeup. One of these policies is to avoid the interbreed mating and loss of original breeds. Genetic

diversity consists of inter and intra breeds which is one of the main tools of animal breeding and genetic improvement. Genetic diversity between populations and also their similarities are the results of different processes which have been achieved over years. It is very important for each country to save these variations and proper strategies are made whenever accurate information from genetic structure of livestock is available. One of the tools for detecting these characteristics is genetic markers. Genetic distances among all breeds of one species and e resulted phylogeny may help us in making proper decisions about selection policies and studying the evolutionary trends. There distances are best indicators of genetic relations between different breeds. Since distance criteria are deficient in determining the influences of artificial selection on economic traits, they may be used only as a primary guideline for studying the natural structures and breed differentiations. In making final decisions for breed selections, one must consider all information available about economic value, basic structure, the existence of certain genes and phenotypes, local or regional importance of certain breeds and availability of resources.

As a conclusion, the three different populations of Sarabi, Sangsari and Afshari dogs are separate populations regarding their genetics. This diversity indicates that these populations have not been crossed to each other which in comparison to other animals like sheep which has been affected by human migration, is a favorable situation.

These differences between populations of dogs shows that there must not be a unique policy for all populations of dogs and breeding plans for each one must be chosen separately.

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