

Microsatellite loci analysis for the genetic variability and paternal lineages in Iranian native dogs

M. Montazery, A. A. Masoudi* and R. Vaez Torshizi

Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

* Corresponding author, E-mail address: masoudia@modares.ac.ir

Abstract Genetic variation among the individuals is considered as an important tool for conservation of livestock animals. This study was conducted to analyze the genetic variation, phylogenetic relationship and paternal lineages among Iranian dog populations using seventeen autosomal and Y chromosome-specific microsatellite markers. Total DNAs of the samples were extracted and applied for genotype analysis. The results demonstrated that all populations except the Bakhtiari, Sarabi and Alborz samples showed significant deviations from Hardy–Weinberg equilibrium (HWE) at more than one locus. The number of alleles per population ranged from 4 (FH3053) to 9 (FH20609,20) with a mean of 6.2. In addition, based on the effective alleles, Shannon index and PIC, FH3053 and FH20609, 20 loci indicated the least and the most diversity. The expected heterozygosity varied from 0.53 in Sangsari population to 0.72 in Kurdish population. The highest and lowest genetic distance were detected between Tazi and Alborz and between Bakhtiyari and Sarabi populations, respectively. Y-Specific markers identified eight paternal haplotypes in the populations, indicating various paternal lineages in Iranian native dogs. The results of the current study indicated the segregation of breed-specific alleles in some dog populations, and showed that the populations had a relatively high genetic variation. The findings could be useful in the designing of breeding strategies and conservation of the dog populations in Iran.

Keywords: haplotype, Y-chromosome, microsatellite marker, heterozygosity, Iranian dogs

Received: 07 Apr. 2015, accepted: 27 Apr. 2016, published online: 04 May. 2016

Introduction

Domestic dog (*Canis familiaris*) belongs to the order Carnivora, and is originated from their carnivorous ancestors and probably gray wolf in the past (Bannasch et al., 2005). The dog has been one of the companions of human from ancient time until now and has been used for various purposes such as guarding and hunting. Over thousands of years, the species has evolved into different breeds with various behavioral and physiological characteristics (Parker et al., 2004). Breeding of the domestic dog provides an unparalleled opportunity to explore the genetic diseases as well as morphological variations and behavioral characters. The position of the dog within the mammalian evolutionary tree also makes it an important guide for comparative analysis of the human genome (Lindblad et al., 2005).

From animal breeders' standpoint, the knowledge of the genetic structure of populations can be of great help to preserve genetic resources (Kukekova et al., 2009). It has been shown that genetic diversity of animals is useful in population and its lack or shortage reduces the strength of future selections. Therefore, the knowledge

of genetic diversity is important for reformers to maintain the genetic resources and develop the genetic strategies for next generations (Jack and Felix, 1996). Furthermore, it would be helpful to reduce the risk of reduction of the animals in nature and prevent their extinction, creating the opportunity for oncoming new markets and use the native gene pool for saving the quantitative or qualitative and fitness related traits in a population. Reduction of the genetic diversity enhances the risk of inbreeding in which consequently pave the way to fix some specific alleles within a breed and then result in increasing the hereditary defects in the population (Kang et al., 2009).

The domestic dog shows great variations in morphology and behavior which make it an interesting subjects for scientific studies (Kukekova et al., 2009). In recent years, various studies have been designed by genetic markers that have helped in separating the species and could be used in breed preservation programs. Moreover, these markers are useful in determining the genetic diversity between or within populations (Lindb-

lad et al., 2005). The most available and efficient markers, microsatellite markers, have been developed and are being used in population diversity and conservation studies or mapping the canine genome (Parker et al., 2004; Kang et al., 2009).

In addition to autosomal markers which are able to show genetic diversity in different populations (Bannasch et al., 2005), the application of Y chromosome markers also can be used to determine the paternal lineages and male diversity within or between populations. Because male and female animals do not play the same role in population structure, this asymmetry between the sexes affect the breeding strategies (Greenwood, 1980). The effect of male offspring on inbreeding is greater than that of the females, therefore, male animals have a significant role in creating pure samples in the population, which decreases the variation among animals. Low variation within the population leads to inbreeding depression. Since the Y chromosome is passed only through male animal to the next generation and it shows low recombination, the study of this chromosome is useful to follow the derivation of origin of the sire lineage of animals. The results could be applied in designing the mating systems to increase the variation within or between populations.

Few studies on Y chromosome, using a specific regional sequence or specific microsatellite markers, have been carried out to uncover the genetic structure and paternity lineages of animals (Sundqvist et al., 2001; Bannasch et al., 2005; Ding et al., 2012). Moreover, the Y-chromosome markers in combination with autosomal chromosomes have been used for better understanding of the dog-wolf genetic analysis and hybridization (Iacolina et al., 2010).

Archeological findings have indicated the presence of dogs in Iran as early as 6000 BC (Dadvar and Khosravifar, 2012). Dog rearing in Iran has been strongly influenced by the environments within which they have been raised, from the rough terrain and hilly regions in the northwest to dry and harsh conditions in the south or east. This has resulted in the development of discrete populations of dogs with distinct genetic characteristics such as herding, guarding and hunting. Iranian native dog populations could be divided to several ecotypes, according to physical appearance and habitats.

In spite of the presence of a range of diverse indigenous dog ecotypes in Iran, genetic studies on dogs are not well established. Therefore, the current study was recommended by the Research Center of Antinarcotic Police of Iran to analyze the genetic structure, variation, distance and the paternal origin and diversity between

Iranian native dog populations. The findings could be applied not only for understanding the structure and relatedness of the animals, but for designing the breeding strategies to protect the effective size of the populations.

Materials and methods

Sample collection

A total of 97 blood samples were collected randomly from 5 dog ecotypes, namely, Sarabi (n=20), Kurdish (n=12), Bakhtiari (n=15), Tazi (Saluki) (n=8) and Sangsari (n=6), and 3 free populations of Western part (n=11), Khorasan (n=10) and Alborz (n=15). These populations are distributed across various parts of Iran. The Tazi (Saluki) ecotypes were widespread along the Silk Route from east to west and were used as good hunters due to its high flexibility and quickness in running. The Sarabi ecotype is considered one of the oldest and powerful indigenous dogs in Iran which is used for guarding and fighting. These two dogs are native of East Azerbaijan province. Kurdish dog is a large- ecotype originating from the West Azarbaijan., Kurdistan and Kermanshah provinces. Other Iranian dog ecotypes, including the Bakhtiari and Sangsari, are commonly used for herding.

DNA extraction and microsatellite markers

The DNA was extracted using the phenol-chloroform method (Paabo et al., 2004). A total of 17 microsatellite markers, including 13 autosomal markers and 4 Y-chromosome-specific markers, from unconserved genome, were used in this study (Table 1). Microsatellite markers were chosen on the basis of polymorphic information content (PIC) and heterozygosity values reported in earlier studies (Richman et al., 2001; Guyon et al., 2003; Breen et al., 2001; Mellersh et al., 2000; Bannasch et al., 2005; Olivier et al., 1999). The PCR reaction was accomplished in a total volume of 15 μ L using 30 ng of genomic DNA, 2 mM $MgCl_2$, 2.5 mM dNTPs, 1.5 μ L PCR buffer (10x), 10 pmol of each primer and 1 μ L of Taq polymerase. The amplifications were carried out using a denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing (44-60°C) for 30 s, extension at 72°C for 30 s and a final extension for 5 min at 72°C. Then, 7 μ L of amplified product was mixed with 4 volumes of formamide loading dye and denatured at 95°C for 5 min and separated on a 6% polyacrylamid gel for 14 h. The gels were silver stained and the bands were visually observed on the gels. The discrimination of the microsatellite alleles was determined by comparison with an adjacent DNA ladder.

Table 1. Information on microsatellite markers used in this study

Marker	Forward Primer 5' – 3'	Reverse Primer 3' – 5'	Annealing Temperature (°C)	Cho. N.	Reference
C26.73320	CCCTCTACTTATGTCTCGGCC	GAGAGGAGAAACAACCAACACC	56	26	Richman et al., 2001
CXX.6727,10,18	AGGTCAGGGACAAATATACCGT	CCTGAGCCCCAAGGCAGAC	56	23	Richman et al., 2001
FH20609,20	GTTTTGAGGAAGCCTTGCTG	GAAGGAAGGGGCCAGTATTTC	58	8	Richman et al., 2001
FH20169, 20	CATTTTAAAGGATGGAGACAGC	AACAGTGTCCCATGGCCTAC	57	1	Richman et al., 2001
FH2790	AGGCCCTTCTCTGTCTCTCTTG	CCAATATTGTTAAGAAAGTTCAAC	57	33	Guyon et al., 2003
FH2914	GTGATCCACTTGCTTGTATCC	ATAGCCCTGGGAAATTTTTCG	56	21	Guyon et al., 2003
FH3053	GATTAAAAAGGCAAGCAACC	TCATCTCCAGCTTTTCATGG	54	30	Guyon et al., 2003
FH2795	TGTGAGTAGGGTAGGGCAGG	GGCTGGACITTTTGTCATTTC	56	18	Guyon et al., 2003
REN86G15	CAACTAAGGCAGAGAATACCA	CAGCGAATTTGGGCACATA	56	38	Breen et al., 2001
REN144M10	TCTCATGCAAAATCTTCGTTCA	GCCACCTCATTCCAA AAAAGA	53	15	Breen et al., 2001
REN87O21	TGGCTGTGGCTAAGGCTTTGT	CCCCCTCAGCTTCGGGTGAG	57	26	Breen et al., 2001
REN126A15	CTACAGGGCAGTCCACTCTT	AAAGGGATTCAGGGAGGTTTG	57	9	Breen et al., 2001
REN59H07	AGGCACTTATCGCTCTCAT	TTGTTTTTCCATCATTTTT	46	4	Mellersh et al., 2000
650-79-2	ACTCAGCTCTCCCTTGTCCTCA	TTGAGGGCAGGTCCTCAGAGT	60	Y	Bannasch et al., 2005
650-79-3	AGTTTCTGCCCCAGGAAGGAC	AGCTGAGCGGTTTGAAAACCTT	60	Y	Bannasch et al., 2005
SRY	GAACGCAATCTTGGTGTGGTCTC	GGCCATTTTTTCGGCTTCTGTAAAG	60	Y	-
AF192268	GCCCTGGAAAGTGATATATTCCTCTAA	TCTGAAGCTTCTTGTATGTGTCTCAG	60	Y	Olivier et al., 1999

Statistical analysis

The size of alleles, allele types and kind of genotypes were determined by the Excel software. The PopGene1.31 Software was used for estimation of the Hardy–Weinberg equilibrium and diversity criteria such as, allele frequencies, the mean number of alleles per locus, observed heterozygosity (H_o), expected heterozygosity (H_e), and Shannon index for each locus (Yeh et al., 1999). The Shannon index criterion is very useful for the measurement of genetic diversity of the loci that have high polymorphism, like microsatellite markers (Alipor et al., 2013). The PIC was calculated by the Het Software (Ott, 1992).

The genetic relationships among Iranian native dogs were analyzed using two different approaches based on allele frequencies at microsatellite loci: first, pair-wise F_{ST} was used to establish genetic distances among the breeds under genetic drift, which is the main factor promoting genetic differentiation between the breeds; second, the genetic distance between the breeds was calculated according to Nei's genetic distance (Nei, 1972). The former was analyzed using GeneAlex 6.5 (Peakal and Smouse, 2006), and the latter was calculated by Pop-Gene1.31 Software (Yeh et al., 1999). Phylogenetic trees were constructed using neighbor-joining (NJ) clustering (Nei, 1972) from Nei's distance method.

The number of Y-chromosome microsatellite haplotypes in different populations were used as a measure of genetic diversity. Because mutations in microsatellite loci are normally produced by the addition or deletion of one or two repeat units (Ellegren, 2004), two haplotypes that differ at multiple microsatellite loci or by multiple repeat units are not likely to have recently been derived from each other. Haplotypes were constructed

based on the presence of alleles for the four Y-chromosome microsatellites, and allele and haplotype diversity were calculated using Arlequin 3 software (Excoffier et al., 2007).

Results

Genetic diversity in Iranian native dogs

The allele and genotype frequencies of 13 microsatellite loci were determined in 8 Iranian native dog populations. Some characters of the microsatellite loci in all animals are shown in Table 2. All microsatellite markers used in the current study showed polymorphism, except the FH20169,20. Although some unique alleles were found in certain populations, these are unlikely to be useful as population specific markers due to their low frequency in the populations.

Seventy six alleles were identified across the 13 microsatellite markers. The total number of alleles per locus varied from 4 (FH3053) to 9 (FH20609,20). The average allele number of polymorphic loci was 6.2 allele per locus. The highest and the lowest effective alleles ranged from 5.93 (FH20609,20) to 2.44 (FH3053) among the populations. The heterozygosity was used to investigate the diversity and effectiveness of genetic marker. The highest rate of intended heterozygosity was related to FH20609, 20 locus (0.83) and the lowest to FH3053 locus (0.59). The highest and lowest Shannon indexes were observed in FH20609,20 locus (1.81) and FH3053 locus (1.06), respectively. These values seem quite reasonable in comparison to the allele numbers of these two loci.

The F_{ST} value for each locus is very close and levels of apparent breed differentiation were considerable (Table 2). Multi locus F_{ST} values indicated that approxima-

Table 2. The criteria diversity of all animals

Loci	No. of Alleles	No. of effective alleles	Shannon Index	PIC	F_{IS}	F_{IT}	F_{ST}	H_e	H_o^*
C26.73320	7	3.72	1.41	0.70	0.49	0.63	0.23	0.73	0.24
CXX.6727,1 0,18,	5	3.34	1.32	0.63	0.58	0.64	0.16	0.71	0.24
FH20609,20	9	5.93	1.81	0.83	0.46	0.59	0.18	0.83	0.32
FH2790	6	4.78	1.65	0.75	0.29	0.43	0.16	0.79	0.45
FH2795	6	3.23	1.42	0.61	0.68	0.72	0.09	0.69	0.19
FH2914	5	4.38	1.53	0.68	0.57	0.68	0.14	0.77	0.24
FH3053	4	2.44	1.06	0.46	0.30	0.41	0.15	0.59	0.30
REN59H07	6	4.34	1.50	0.63	0.58	0.65	0.18	0.77	0.27
REN87O21	5	3.38	1.33	0.61	0.91	0.93	0.21	0.70	0.05
REN126A15	8	4.50	1.62	0.79	0.54	0.59	0.09	0.78	0.29
REN144M10	7	5.14	1.72	0.77	0.48	0.56	0.12	0.80	0.36
REN86G15	8	4.94	1.7	0.79	0.40	0.52	0.14	0.80	0.32
Mean	6.2	4.4	1.5	0.68	0.52	0.61	0.15	0.73	0.27

*All breeds examined, PIC: Polymorphic Information Content, H_e : Expected Heterozygosity, H_o : Observation Heterozygosity

Table 3. Sample size, allele diversity (the mean number of observed alleles per locus), observed heterozygosity (H_o), expected heterozygosity (H_e) and P values of Iranian dog populations across all loci

Populations	Sample Size	Allele Diversity	H_o	H_e	P-val. (No. of Loci) ^a
Tazi (Saluki)	8	3.8	0.36	0.64	0.084 (2)
Khorasan samples	10	3.5	0.30	0.56	0.040 (2)
Sangsari	6	3.0	0.26	0.53	0.010 (3)
Kurdish	12	5.1	0.29	0.72	0.081 (2)
Western part samples	11	3.4	0.27	0.61	0.032 (2)
Bakhtiari	15	4.5	0.23	0.66	0.031 (1)
Sarabi	20	4.5	0.28	0.68	0.025 (1)
Alborz samples	15	4.0	0.25	0.59	0.038 (1)
Average	12.1	3.9	0.28	0.62	0.042 (14)

^aNumber of locus showing departure from Hardy–Weinberg equilibrium.

tely 15% of the total genetic variation was explained by breed differences, with the remaining 85% corresponding to differences among individuals. On the average, breeds had a 5.2% deficit of heterozygotes, whereas the total population had a 61% deficit of heterozygotes.

All populations, except the Bakhtiari, Sarabi and the Alborz samples, showed a significant deviation from HWE at more than one locus (Table 3). Out of 96 cases (8 populations, 12 loci), 14 loci (locus FH20609, 20 in Sarabi, loci FH3053 and FH2914 in Western area., loci FH3053 and C26.73320 in Khorasan, loci FH3053 and FH20609, 20 in Tazi (Saluki), loci FH3053 and CXX.6727,10,18 in Kurdish, loci REN86G15, REN144M10 and REN59H07 in Sangsari, locus FH2790 in Alborz and locus FH3053 in Bakhtiari populations) showed significant deviations from HWE ($P < 0.05$). The maximum and minimum of inter population diversity, which is calculated as the average intended heterozygosity in all loci for every population, were obtained from Kurdish (0.72) and Sangsari (0.53) populations, respectively.

Genetic relationships among the Iranian native dogs

Table 4 shows the Nei's genetic distance, F_{ST} , and

N_m values when were are considered in couples. Across the 12 loci, the mean genetic differentiation values (F_{ST}) ranged from 0.04 for the paired population of Sarabi-Bakhtiari to 0.15 for the Tazi (Saluki)-Alborz. The number of migrants per generation (N_m) varied from 1.5 for the Tazi (Saluki)-Alborz to 5.17 for the Sarabi-Bakhtiari paired populations, indicating high gene flow between the Sarabi and Bakhtiari dogs (Table 4). It should be noted that the gene flow among the Khorasan dogs seems to be relatively high in comparison to the other dog populations. When genetic data are obtained from several populations to investigate genetic similarity, genetic distance matrix is used. The smallest genetic distance (0.31) was observed between the Sarabi and Bakhtiari populations, and the largest (0.85) was detected between the Tazi (Saluki) and Alborz samples (Figure 1; Table 4). The Alborz population showed the highest levels of between-population divergence ($F_{ST} = 0.06$ to 0.15 and Nei's distance = 0.37 to 0.85) in comparison to other populations.

Analysis of Y chromosome microsatellite markers in male animals

To analyze the locus-specific markers from the Y chromosome, four microsatellite loci were genotyped in 25

Table 4. The Nei's genetic distance, mean F_{ST} estimates, and migrants per generation (N_m) values between each pair of 8 Iranian dog populations

Populations	Sarabi	Bakhtiari	Alborz samples	Kurdish	Khorasan samples	Western part samples	Tazi (Saluki)	Sangsari
Sarabi	-	0.04 (5.17)	0.08 (2.63)	0.11 (2.02)	0.06 (3.73)	0.10 (2.19)	0.08 (2.79)	0.08 (2.73)
Bakhtiari	0.31	-	0.06 (3.43)	0.11(2.00)	0.06 (3.89)	0.11 (2.02)	0.08 (2.82)	0.09 (2.34)
Alborz samples	0.58	0.37	-	0.10 (2.25)	0.08 (2.76)	0.13 (1.57)	0.15 (1.50)	0.14 (1.35)
Kurdish	0.35	0.44	0.55	-	0.09 (2.47)	0.12 (1.76)	0.08 (2.78)	0.13 (1.58)
Khorasan samples	0.58	0.5	0.61	0.80	-	0.06 (3.66)	0.06 (3.61)	0.06 (3.83)
Western part samples	0.38	0.42	0.75	0.34	0.47	-	0.09 (2.40)	0.11 (1.99)
Tazi (Saluki)	0.42	0.62	0.85	0.33	0.62	0.53	-	0.10 (2.17)
Sangsari	0.54	0.61	0.45	0.48	0.52	0.68	0.44	-

Above the diagonal, F_{ST} values and N_m (between bracket) values; below the diagonal, genetic distance values are given.

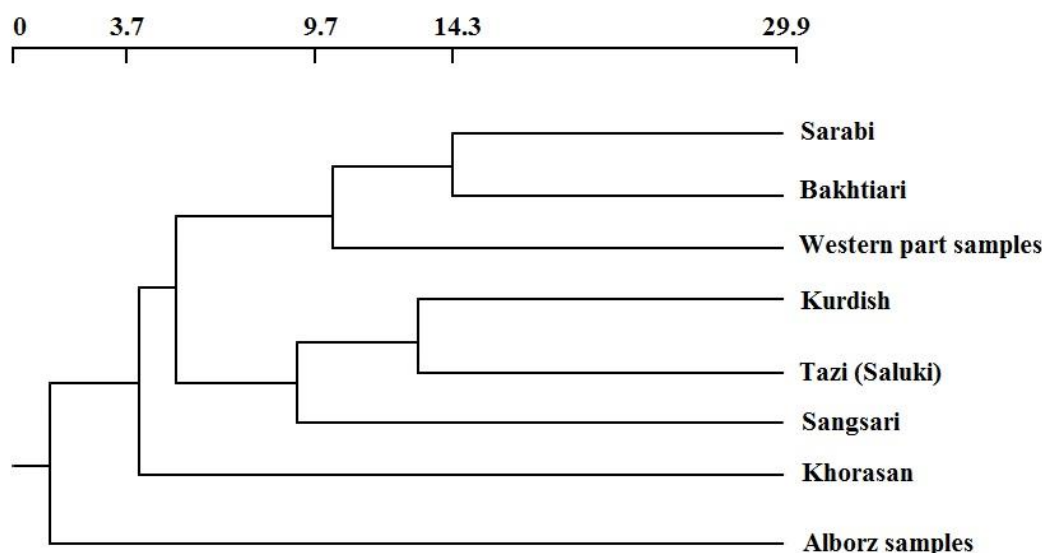


Figure 1. The genetic relationships among 8 populations of Iranian native dogs, based on the Nei's distances. Values along the branches represent bootstrap support in percent from 1000 replications of resampled loci. The scale indicates genetic distance.

males from Iranian dog populations. All markers, except 650-79.3, were polymorphic, having two to four alleles. In a preliminary analysis of Y chromosome haplotype diversity among Iranian native dogs, 8 different haplotypes (A, B, C, D, E, F, G, and H) were found (Table 5). Haplotype F showed the highest frequency (7) among the haplotypes. Haplotypes C-D and haplotypes E-F differed only in the genotypes of the locus AF192268,

and A-G differed only in the genotypes of the locus 650-79.4. Haplotypes B and H showed variation at loci AF192268 and 650-79.4. Haplotypes D and E were observed in Sarabi population but haplotype F was only found in Khorasan samples (Table 6). The number of haplotypes found per populations varied from 1 (Tazi) to 5 (Sarabi). The Tazi (Saluki) showed the lowest haplotype diversity.

Table 5. Y-chromosome microsatellite haplotypes found in the Iranian native dogs and allele sizes (bp) at four microsatellite loci

Haplotype	A	B	C	D	E	F	G	H
Locus								
650-79.3	150	150	150	150	150	150	150	150
650-79.4	144	138	144	144	150	150	128	128
SRY	138	140	140	140	140	140	138	140
AF192268	212	212	212	218	218	212	212	218

Table 6. Distribution of Y-chromosome microsatellite haplotypes across the male dogs

Population Haplotype	Tazi (Saluki)	Sarabi	Khorasan samples	Bakhtiari	Western part samples	Total
A	5	1	-	-	-	6
B	-	1	-	1	-	2
C	-	1	1	-	-	2
D	-	1	-	-	-	1
E	-	3	-	-	-	3
F	-	-	7	-	-	7
G	-	-	-	1	1	2
H	-	-	-	1	1	2

Discussion

Because of the gene flow among the dog populations, analyzing the genetic relationships of dog breeds could be difficult. Evaluation of frequency differences in microsatellite markers may be used for differentiation analysis of closely related species (Kim et al., 2001; Kang et al., 2009). In the current study, using the thirteen autosomal and four Y-linked microsatellite loci, the diversity, genetic structure and genotype distributions were analyzed in eight populations of Iranian native dogs.

All dog populations showed significant deviation from HWE. Genetic drift and division of the populations to smaller sub-populations over many generations is likely to cause changes in their genetic structure. In addition, lack of a control in exchange of genetic material in different areas, decreasing the number of native animals in each geographical region, uncontrolled mating and the limited number of samples could also affect the deviation of the populations from HWE. Deviation from HWE is being observed in some dog populations (Zajc et al., 1997; Kang et al., 2009; Kim et al., 2001). Moreover, greater genetic homogeneity and bottlenecks associated with catastrophic events such as war and economic depression could be observed in domestic dogs, which resulted in some deviations from HWE (Zajc et al., 1997). The microsatellite loci with 4–9 alleles per loci showed high variability in these populations. In the present study, six microsatellite markers (FH20609, 20, REN144M10, REN86G15, REN126A15, FH2790 and C26.73320) showed high polymorphism in all populations indicating a great potential for parental testing and population-based genetic studies.

One of the most common criteria of molecular markers for the evaluation of the allelic diversity is heterozygosity. Our results indicated that the heterozygosities in these populations ranged from 0.53 (Sangsari) to 0.72 (Kurdish), indicating a strong contribution of different individuals in matings. The expected heterozygosities in the Iranian dog populations are similar to those of Asian dogs (0.54, Kishu; 0.69, Jindo; 0.59, Eskimo and 0.71, HAD) reported by Kim et al. (2001) and European dogs (0.52, English Setter; 0.65, Epagneul Breton and 0.55, German shepherd) reported by Parra et al. (2008), and Cho and Cho (2003). The Sangsari showed the lowest average allele number as well as the lowest heterozygosity, showing a considerable reduction in inter-population variation and extensive inbreeding. In addition, the low diversity in this population could be due to the reduction in the number of the dogs in its native region

and the small sample size. High variation in Kurdish population might be related to the sampling of the animals from a vast geographical region in which they are spread. Gene flow, an evidence of gene exchanges between populations, has an important role in gene frequency changes within and between populations. The F_{ST} statistics and Nei's genetic distance of the breed relationships and evolutionary studies are applied in comparing of the genetic values of the desirable animals. The highest genetic distance was observed between Tazi (Saluki) and Alborz samples (0.85) and Khorasan and Kurdish samples (0.80). It should be noted that the geographical locations of the animals are far from each other and migration among populations is less likely to have occurred. On the other hand, specific physical characters of these populations resulted in the reduction of the breeding between animals and consequently reduced the gene fusion between the populations. The dendrogram of the relationships between the samples showed a low genetic distance between Sarabi and Bakhtiari samples which is concordant with the evidences on the close relatedness of these two populations.

Autosomal microsatellite markers indicated a high genetic divergence in the Iranian native dogs (F_{ST} = 15%), which are similar to the Asian dogs (F_{ST} = 15.4%) reported by Kim et al. (2001), but lower than those reported by Parker et al. (2004) who analyzed 85 breeds of dogs and found a value of 27% for F_{ST} . However, the F_{ST} value in European dog breeds ranged from 0.099 (Jordana et al., 1992) to 0.108 (Morrena et al., 1999). The results might support the hypothesis of the origin of the dog from a single place in southeastern Asia in the Old World, and migration of some of the haplotypes to Europe or other parts of the world (Ardalan et al., 2011; Ding et al., 2012). During migration, a limited number of haplotypes succeed to pass the origins in the ancient world to reach Europe.

Subsequently, to understand the paternal lineages of the dog population in Iran, Y-chromosome microsatellite analysis was applied to available male animals. Although a small portion of the samples were male animals, 8 different haplotypes were observed. The results indicated that haplotype A in Tazi (Saluki) and Sarabi populations, haplotype B in Sarabi and Bakhtiari, haplotype C between Sarabi and Khorasan samples and haplotypes G and H in Western part and Bakhtiari populations were common, therefore, similar parental lineages are observed in Iranian dog populations. Y-chromosome common microsatellite haplotypes in Shepard and Border Collie also indicated a common origin for

paternal lineages of these dogs during the past decades (Bannasch et al., 2005). Our data are in line with the data of Sundqvist et al. (2001) who detected 11 Y-chromosome microsatellite haplotypes in different dog breeds. The paternal lineages of the majority of Iranian dog populations likely originated from a vast number of males in the past; however, to confirm these findings, more samples must be assessed. The highest genetic diversity of Y chromosome was found in Sarabi ecotype. The exact reason for this high degree of diversity is not known, but the habitat of Sarabi dogs is adjacent to that of foreign dogs such as Ghafghazi dogs, which might have introduced some new paternal lineages into the population in the past. On the other hand, the Tazi (Saluki) ecotype showed only one Y-chromosome haplotype; the special features of the animal resulted in avoiding cross-mating with other dog populations. These animals had been kept for hunting by Iranian kings during the past centuries, which resulted in a special attention in treatment of Saluki dog.

Conclusions

Since the mutations in microsatellite loci usually occur through insertion or deletion of one or two repetitive units, it may be concluded that the difference in genetic diversity of the animals is a reflection of differences in genetic coverage. Although numbers of these populations have significantly decreased there is still enough genetic diversity for use in breeding programs and appropriate matting plans to preserve the populations. The existence of the remarkable genetic diversity in Iranian dog samples may be due to the location of Iran on Silk Road in the past. Transportation of animals through this road was usual and this animal because of its marked role in human societies and trades has been introduced to ancient Iran in past centuries. These findings, in addition to the partnership that exists between human and dog, and their migration could be helpful in determining human migration history in the Middle East.

Acknowledgements

The authors wish to thank the Research Center of Anti-Narcotics Police of Iran for supporting this project. Also we would like to thank Jalil Tavanay, Ebrahim Ghareghani and Damon Allahyar Khan Khorasany for their help in collecting the samples.

References

Alipor, A., Darafshan, S., Ghasemi, S.A., 2013. A comparative study of genetic diversity in *Oncorhynchus mykiss* population from Lorestan province with import population from France. *Journal of Natural Research* 66, 199-209.

Ardalan, A., Kluetsch, C.F.C., Zhang, A., 2011. Comprehensive study of mtDNA among Southwest Asian dogs contradicts independent domestication of wolf, but implies dog-wolf hybridization. *Journal of Ecology and Evolution* 13, 373-385.

Bannasch, D.L., Bananasch, M.J., Ryun, J.R., Famula, T.R., Pedersen, N.C., 2005. Y chromosome haplotype analysis in purebred dogs. *Mammalian Genome* 16, 273-280.

Breen, M., Jouquand, S., Renier, C., Mellersh, C.S., Hitte, C., Holmes, N.G., Chéron, A., Suter, N., Vignaux, F., Bristow, A.E., Priat, C., McCann, E., Andre, C., Boundy, S., Gitscham, P., Thomas, R., Bridge, W.L., Spriggs, H.F., Ryder, E.J., Curson, A., Sampson, J., Ostrander, E.A., Binns, M.M., Galibert, F., 2001. Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome Research* 11, 1784-1795.

Cho, G.J., Cho, B.W., 2003. Validation of microsatellite markers for routine canine parentage testing in Korea. *Genetics* 25, 103-108.

Dadvar, A., Khoravifar, S.H., 2012. A comparative study on the role of hunting in Iranian ancient art and Beinhlohnrein art. *Archive of SID* 1, 29-44.

Ding, Z.L., Oskarsson, M., Ardalan, A., Angleby, H., Dahlgren, L.G., Tepeli, C., Kirkness, E., Savolainen, P., Zhang, Y.P., 2012. Origins of domestic dog in Southern East Asia is supported by analysis of Y-chromosome DNA. *Heredity* 108, 407-514.

Ellegren, H., 2004. Microsatellite: simple sequences with complex evolution. *Genetics* 5, 435-446.

Excoffier, L., Laval, G., Schneider, S., 2007. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1, 47-50.

Greenwood, P.J., 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behavior* 28, 1140-1162.

Guyon, R., Lorentzen, T.D., Hitte, C., Kim, L., Cadieu, E., Parker, H.G., Quignon, B.P., Lowe, J.K., Renier, C., Gelfenbeyn, F.O., DeFrance, H.B., Gloux, S., Mahairas, G.G., Andre, C., Galibert, C., Ostrander, E.A., 2003. A 1-Mb resolution radiation hybrid map of the canine genome. *Proceedings of the National Academy of Sciences* 100, 5296-5301.

Iacolina, L., Scandura, M., Gazzola, A., Cappai, N., Capitani, C., Mattioli, L., Vercillo, F., Apollonio, M., 2010. Y-chromosome microsatellite variation in Italian wolves: A contribution to the study of wolf dog hybridization patterns. *Mammalian Biology* 75, 341-347.

Jack, E.S., Felix, C.S., 1996. Genetic marker map construction and their application in plant breeding. *Hortscience* 31, 104-109.

- Jordana, J., Piedrafit, J., Sanchez, A., Puig, P., 1992. Comparative F statistics analysis of the genetic structure of ten Spanish dog breeds. *Journal of Heredity* 83, 367-374.
- Kang, B.K., Kim, K.S., Mim, M.S., Chae, Y.J., Kang, J.W., Yoon, J., Choi, J., Seong, J.K., Park, H.C., An, J., Lee, M.H., Park, H.M., Lee, H., 2009. Microsatellite loci analysis for the genetic variability and the parentage test of five dog breeds in South Korea. *Genetics* 84, 245-251.
- Kim, K.S., Tanabe, Y., Park, C.K., Ha, J.H., 2001. Genetic variability in East Asian dogs using microsatellite loci analysis. *Journal of Heredity* 92, 398-403.
- Kukekova, A.V., Vorobieva, N.V., Beklemisheva, V.R., Johnson, J.L., Temnykh, S.V., Yudkin, D.V., Trut, L.N., Andre, C., Galibert, F., Aguirre, G.D., 2009. Chromosomal mapping of canine-derived BAC clones to the red fox and American mink genomes. *Journal of Heredity* 1, 42-53.
- Lindblad, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 438, 803-819.
- Mellersh, C.S., Hitte, C., Richman, M., Vignaux, F., Priat, C., Jouquand, S., Werner, P., Andre, C., DeRose, S., Patterson, D.F., Ostrander, E.A., Galibert, F., 2000. An integrated linkage-radiation hybrid map of the canine genome. *Mammalian Genome* 11, 120-130.
- Morrena, L., Barba, J., Garrido, J., Barbancho, M., Andrés, F., 1999. Genetic variation detected by microsatellites in five Spanish dog breeds. *Journal of Heredity* 90, 654-656.
- Nei, M., 1972. Genetic distance between populations. *American Naturalist* 106, 283-292.
- Olivier, M., Breen, M., Bins, M.M., Lust, G., 1999. Localization and characterization of nucleotide sequences from the canine Y chromosome. *Chromosome Research* 7, 223-233.
- Ott, J., 1992. Program PIC (version 1.51): Utility programs for detection of polymorphism information content. *The American Journal of Human Genetics* 51, 283-290.
- Paabo, S., Poinar, H., Scrrc, D., 2004. Genetic analyses from ancient DNA. *Genetics* 38, 645-679.
- Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A., Kruglyak, L., 2004. Genetic structure of the purebred domestic dog. *Animal Science* 304, 1160-1164.
- Parra, D., Mendez, J., Canon, J., Dunner, S., 2008. Genetic differentiation in pointing dog breeds inferred from microsatellites and mitochondrial DNA sequence. *Animal Genetics* 39, 1-7.
- Peakal, R., Smouse, P.E., 2006. GeneAlex (version 6.5): Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.
- Richman, M., Mellersh, C.S., Ander, C., Galibert, F., Ostrander, E.A., 2001. Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. *Journal of Biochemical and Biophysical Methods* 47, 137-149.
- Sundqvist, A., Greenwood, H., Olivier, M., Vila, C., 2001. Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers. *Molecular Ecology* 10, 1959-1966.
- Yeh, F.C., Yang, R., Boyle, T., 1999. POPGENE. Version 1.31. Microsoft Window- based Freeware for Population Genetic Analysis. University of Alberta. Edmonton, AB, Canada.
- Zajc, I., Cathryn, S.M., Sampson, J., 1997. Variability of canine microsatellites within and between different dog breeds. *Mammalian Genome* 8, 182-185.

Communicating editor: Ali K. Esmailizadeh

آنالیز جایگاه‌های نشانگر ریزماهواره برای بررسی تنوع ژنتیکی و خطوط پدری سگ‌های بومی ایران

م. منتظری، ع.ا. مسعودی^{*}، و ر. واعظ ترشیزی

گروه علوم دامی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.

نویسنده مسئول، پست الکترونیک: masoudia@modares.ac.ir

چکیده تنوع زیستی یکی از عوامل مهم برای اصلاح‌گران حیوانات اهلی جهت حفظ این ذخایر ژنتیکی محسوب می‌شود. در این تحقیق به بررسی تنوع ژنتیکی، رابطه فیلوژنتیکی و خطوط پدری جمعیت سگ‌های بومی ایران، با استفاده از ۱۷ نشانگر ریزماهواره‌ای اتوزومی و نشانگر مختص کروموزوم Y پرداخته شد. کل DNA نمونه‌ها، استخراج و برای تعیین ژنوتیپ بکار گرفته شد. نتایج بدست آمده حاکی از آن بود که همه جمعیت‌ها به جزء بختیاری، سرابی و البرز در یک جایگاه یا بیشتر، انحراف معنی‌داری از تعادل هاردی-واینبرگ را نشان دادند. ($P < 0.05$) دامنه تعداد آلل در جمعیت‌ها از ۴ (FH3053) تا ۹ (FH20609,20) بود (با میانگین ۶/۲). هم‌چنین مطالعه تعداد آلل موثر، شاخص شانون و معیار PIC نیز نشان داد که جایگاه FH3053 و FH20609,20 به ترتیب دارای کمترین و بیشترین تنوع در بین جایگاه‌های مورد مطالعه می‌باشند. بیشترین میزان هتروزیگوسیتی در جمعیت کردی (۰/۷۲) و کمترین آن در جمعیت سنگسری (۰/۵۳) مشاهده شد. هم‌چنین بیشترین فاصله ژنتیکی بین جمعیت‌های بومی البرز و تازی و کمترین آن بین جمعیت‌های سرابی و بختیاری مشاهده گردید. آنالیز نشانگرهای مختص کروموزوم Y نشان دهنده وجود ۸ هاپلوتیپ پدری در جمعیت‌های مطالعه شده بود که نشان دهنده تنوع بالا در این جمعیت‌هاست. نتایج این تحقیق نشان داد که نشانگرهای مورد بررسی در برخی از جمعیت‌های تحت مطالعه حاوی آلل‌های جدیدی می‌باشند. این یافته‌ها می‌تواند برای استراتژی‌های اصلاح نژادی و حفاظت جمعیت‌ها مفید واقع شوند.