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Effects of Habitat Fragmentation on the Genetic Structure of Populations of Wild Goat, *Capra aegagrus* Erxleben, 1777 (Artiodactyla: Bovidae) in Markazi Province, Central Iran

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Abstract: In Markazi Province, Iran, the wild goat (*Capra aegagrus*) is a species of great conservational importance. Habitat fragmentation due to human activities is one of the major threats for species viability. In the present study, seven microsatellite loci were used in order to evaluate the impact of habitat fragmentation on the genetic diversity of wild goat populations from Haftadgholle, Mouteh, Jasb and Rasvand regions located in Markazi Province. Our results demonstrate deviation from the Hardy-Weinberg Equilibrium in all the four studied populations as well as in the studied gene loci. The heterozygosity deficit was high because of the presence of the null allele in all gene loci. As indicated by the Mode-Shift test, the populations from Haftadgholle and Mouteh regions had no genetic bottleneck in the past as opposed to the populations from Jasb and Rasvand regions. The low inter-population diversity (17%), high intra-population diversity (83%), high genetic differentiation, closeness of F_{ST} index (0.191) to R_{ST} index (0.169), high gene flow (1.061) and non-isolated samples in the phylogeny graph reveal that the isolation of wild goat populations in Markazi Province might have happened in less than 30 years and the habitat fragmentation has not any noticeable impact on its genetic diversity.

Keywords: Genetic bottleneck; Genetic diversity; Habitat fragmentation; Microsatellite; Wild goat

Introduction

At present, the destruction of natural habitats due to human activities is one of the most important threats for the survival of many species. The remaining areas as wildlife habitats are often limited and isolated from each other by unsuitable lands. The habitat fragmentation has many potential destructive effects on the populations residing in these areas, including population confinement and inter-population genetic exchange unfeasibility. This causes increase of endogamy and, at the long term, it enhances the danger of extinction. Different species show variety of reactions to the phenomenon based on their various characteristics and requirements (Malekian 2007). As a consequence of habitat fragmentation, various areas with mosaic geographic structures are formed and particular species are not compatible to them. The main threat for the biodiversity is the habitat isolation: the fragmentation of large habitats into isolated patches is known to endanger biodiversity (Fahrig 2003). The fragmented habitats differ from the genuine habitats for two reasons: firstly, the fragmentation creates a greater amount of margin for the habitat, and, secondly, the centre of each fragmented habitat would be closer to the margin. Consequently, the habitat loses its value significantly (Fahring 2003). Most animal habitats in Markazi Province are destructed, changed and fragmented due to severe developmental, industrial, constructional, agricultural and mining activities. The only intact habitats are those under the management

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of the Environment Protection Organisation, i.e. Haftadqolleh, Jasb, Rasvand, Alvand, Mouteh and Palangdareh regions; however, these are too distant from each other and the entire territory is recognised as fragmented.

The wild goat *Capra aegagrus* Erxleben, 1777, as a symbol of Markazi Province's biodiversity, is one of the important species for the regional wildlife (Ansari 2008). The populations of this species are declining at national and global levels due to various factors including uncontrolled hunting, habitat degradation, competition with livestock and others (Ziaie 2008, Weinberg 2008). The wild goat is included as a threatened species in the IUCN Red List as vulnerable. The studies on habitat and ecological requirements indicate a direct relationship between species and characteristics of their habitats. However, the impact of habitat fragmentation on the genetic features of this species is unknown. The reduction of effective population size leads to the increase of random genetic aberrations, rapid loss of genetic diversity and increase of endogamy rate (Eldridge 2008). A high allelic abundance reveals a high effective population size (Diz 2009).

The present study aims to evaluate the extant populations' genetic diversity of wild goat in Markazi Province, Iran. We attempt to answer the following questions: 1) Are the habitat fragmentation phenomenon and population inaccessibility the main reasons of genetic erosion? 2) Does inter-population genetic exchange take place in this species in the region studied?

Materials and Methods

The study was undertaken from May 2012 to May 2013 and covered an area of 1,536,790 ha in the southern part of Markazi Province. The study zone included several protected areas: Haftadqholleh, 97,437 ha, 2,250 individuals of wild goats; part of Moute's wildlife habitat in Isfahan, 60,000 ha, 1,220 wild goats; Jasb wildlife habitat in Delijan, 17,234 ha, 20 wild goats; Rasvand wildlife habitat in Shazand, 1,067 ha, 10 wild goats; the protected area of Alvand in Khomain, 8,618 ha, no wild goats; the protected area of Palang Darreh in Qom, 31,735 m^2, no wild goats. The lack of wild goat populations in the lattest protected area is due to the interruption of the connecting corridors. The main reasons for the fragmentation in the area are the urbanisation, road construction activities, mining activities and changes of land use.

A total of 110 samples of dung was collected from the Southern Markazi Province: Haftadqolleh – 75 samples, Mouteh – 20 samples, Jasb – ten samples and Rasvand – five samples (Fig. 1). The DNA genome was extracted using the Khedrzadeh's method (Khedrzadeh 2010; see also Adams et al. 2003) and ExtractMaster™ Fecal DNA Extraction Kit (2012). Seven loci were selected based on previous studies (Citation). The loci proliferation was carried out using PCR (Table 1). The PCR products were isolated using 10% acrylamide gel. To determine the allele size, the 50 bp DNA nucleotide marker was used. Then the gels were stained using the silver nitrate method and after taking their images by the Gel Documentation Device, the fragments lengths were calculated using the Gel-Pro Analayzer software, v. 3.1. The null alleles were evaluated using Microchecker software (Van, 2004). Allele frequencies, number of alleles per locus, effective alleles numbers, expected heterozygosity, observed heterozygosity, Shannon index, endogamy, gene flow, inter- and intra-population genetic diversities and inter-population differentiation were determined based on "F_{ST}" infinite allele model and "R_{ST}" step mutation

Table 1. Characteristics of the studied loci

Nucleotide sequences Primers	Junction temperature	Chromosome	Weight(bp)	Loci
F: 5'–TGCGGTCTGGTTCTGATTTCAC–3' R: 5'–CCTGCATGAGAAAGTCGATGCTTAG–3'	55	Unknown	216-244	SRCRSP08
F: 5'–CGGGGATCTGTTCTATGAAC–3' R: 5'–TGATTAGCTGGCTGAATGTCC–3'	55	10	112-132	SRCRSP03
F: 5'–GGACTCTACCAACTGAGCTACAAG–3' R: 5'–TGAAATGAAGCTAAAGCAATGC–3'	55	21	164-190	SRCRSP05
F: 5'–GGAAGCAATGAAATCTATAGCC–3' R: 5'–TGTTCTGTGAGTTTGTAAGC–3'	55	10	178-196	ILSTS005
F: 5'–GCTACAGCCCTTCTGGTTTG–3' R: 5'–GAGCTAATCACCAACAGCAAG–3'	59	6	118-162	BM415
F: 5'–GGGTGTGACATTTTGTTCCC–3' R: 5'–CTGCTCGCCACTAGTCCTTC–3'	62	27	202-210	BM203
F: 5'–GAATTCCCATCACTCTCTCAGC–3' R: 5'–GTTCTCCATTGAACCAACTTCA–3'	64	6	140-158	BM302

model using molecular variance analysis (AMOVA) done with the GenAlex software (Peakall 2006). The Bottleneck software was used to determine the populations with genetic bottleneck (Cornuet 1996). The inter-population phylogeography was obtained through drawing trees and graphs that depicted the isolated population using the Structure software, v. 2.3 (Freeland 2005).

Results

A total of 48 different alleles were obtained at all loci with a weight range of 112-244. At BM415 locus, the highest number of alleles was 11 alleles and the largest area's allele size was 118-162. On the contrary, BM203 and SRCRSP03 had the lowest numbers of alleles (four alleles) and the smallest area with the allele size of 210-202 and 112-132, respectively. In the studied gene loci, the number of effective alleles was lower than the observed alleles indicating the higher frequencies of some alleles in a locus. The total number of the observed alleles and effective alleles was highest at Haftadqolleh and lowest at Rasvand. The highest and lowest observed average heterozygosities were obtained at BM302 and SRCRSP05, respectively, and the highest and lowest expected unbiased mean heterozygosities were at BM415 and BM203 (Table 2).

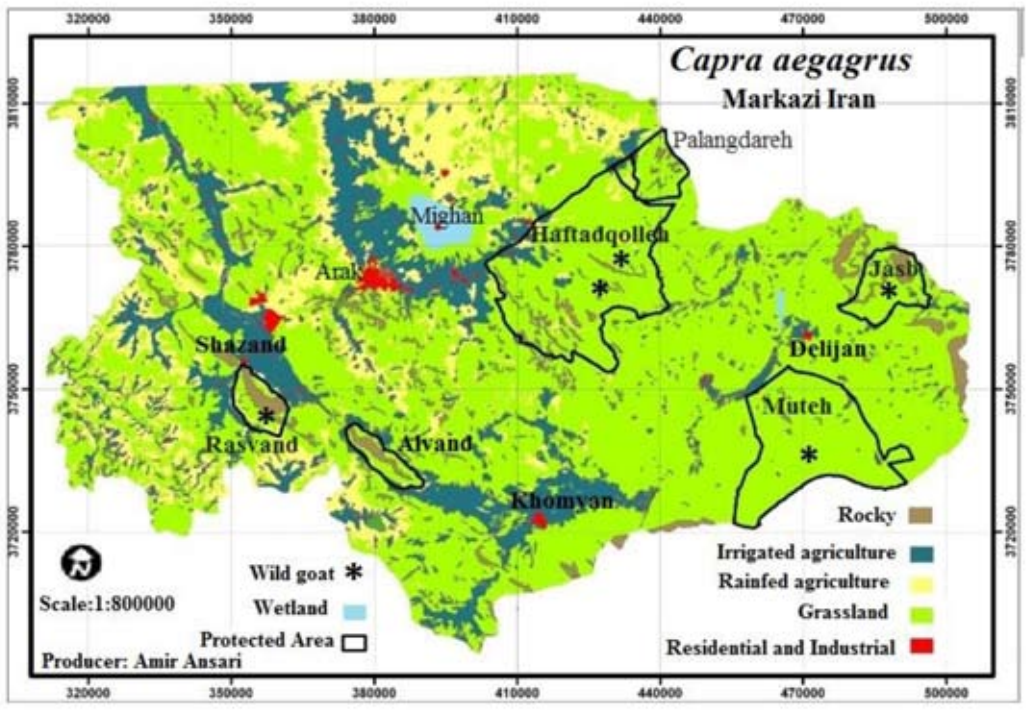

Fig. 1. Sampling area in Markazi Prtovince, Iran

Table 2. Genetic variation of the seven studied loci of populations of wild goat

SRCRSP05	SRCRSP08	BM415	ILSTS005	SRCRSP03	BM203	BM302	Position Average
4	3.5	4.75	3	2.5	2.25	4.75	Na
2.426	2.967	3.786	2.683	1.718	2.007	3.824	Ne
0.016	0.074	0.054	0.029	0.068	0.028	0.194	H_O
0.539	0.578	0.687	0.591	0.405	0.396	0.686	H_e
1.017	0.984	1.322	0.976	0.647	0.641	1.340	I
0.971	0.873	0.921	0.951	0.831	0.930	0.717	Fis
0.256	0.096	0.146	0.094	0.219	0.337	0.186	Fst
0.727	2.341	1.463	2.410	0.891	0.491	1.091	Nm
Imbalance	Imbalance	Imbalance	Imbalance	Imbalance	Imbalance	Imbalance	pHw

Na: number of alleles, Ne: effective number of alleles, HO: observed heterozygosity, He: expected heterozygosity, I: Shannon index, Fis: the stain index, Fst: genetic differentiation, Nm: gene flow, pHw: test probability Hardy – Weinberg.

The highest and lowest expected unbiased mean heterozygosities were observed respectively from Haftadqolleh and Rasvand indicating the high genetic diversity in the population from Haftadqolleh and the low genetic diversity in the population from Rasvand. The highest observed average heterozygosity was obtained from Jasb and Haftadqolleh regions and its lowest value was found in Rasvand Region (Table 3).

The BM415 and BM302 loci showed the highest Shannon values indicating the abundant polymorphism in the loci. Further, the lowest mean value was obtained at BM203 and SRCRSP03 loci (Table 2). The highest and lowest values of Shannon index were observed from the Haftadqolleh and Rasvand regions. The high value of Shannon index in Haftadqolleh revealed the high genetic diversity of the population. These results also corresponded to the mean values of heterozygosity (Table 3).

For Hardy-Weinberg Equilibrium at loci from all regions, the Chi-Square tests and the likelihood ratio showed a deviation from the equilibrium. The endogamy coefficient values in all loci were significant. The mean endogamy coefficient was 0.885. High heterozygosity deficit was observed at the loci (Table 2) and given the results of Microchecker software, its main reason might be the presence of null alleles. Thus, the null alleles were one of the main reasons for the deviation from the equilibrium in these loci. The distinction between the regions was determined using the molecular variance with F_{ST} (0.91) and R_{ST} (0.169) as the distinctiveness indexes. In addition, the results of AMOVA showed that 83% of the observed variance had intra-population origin and only 17% of it was due to inter-population diversity (Table 4).

The maximum value of F_{ST} (0.228) was observed between Mouteh and Rasvand regions while its minimum value (0.063) was determined between Jasb and Rasvand regions with the highest gene flow rate (Nm= 3.743). The lowest gene flow (Nm=1.396) was observed between Haftadqolleh and Rasvand regions; the mean gene flow (1.061) was relatively high in the gene loci of the studied population. $F_{ST} >$ 0.25 is a threshold value for the complete isolation of population. In none of our results the value of F_{ST} reached this threshold. The highest genetic distance was observed between Rasvand and Mouteh regions (0.696) and also between Haftadqolleh and Rasvand regions (0.680) while the shortest distance was found between Rasvand and Jasb regions (0.166) with the highest gene flow rate (Table 5). The results of the Bottleneck software indicated that Haftadqolleh and Mouteh populations had not experienced any genetic bottleneck in the past, but the Mode-Shift test results revealed a genetic bottleneck for Rasvand and Jasb populations (Table 6). The results of the Structure software showed that the data belonged to six populations. We found high population fusion based on the drawn phylogenetic graph (Fig. 2).

All the seven loci used in this study showed polymorphic forms. Therefore, it is possible to use them in future genetic studies on the wild goat. The

Table 3. Genetic diversity of the four populations of wild goat

Rasvand	Jasb	Muteh	Haftadqolleh	Population Average
2.143	2.571	3.286	6.143	Na
1.913	2.246	2.521	4.412	Ne
0.000	0.119	0.052	0.094	H_O
0.463	0.540	0.483	0.731	H_e
0.686	0.840	0.886	1.546	I

Table 4. Analysis of molecular variance (AMOVA)

Amount	scale	Percent	Diversity assessment	Sum of squares	Degrees of freedom	Index
		17	1.82	125.81	3	Between populations
0.169	Rst	83	8.99	953.20	106	Within populations

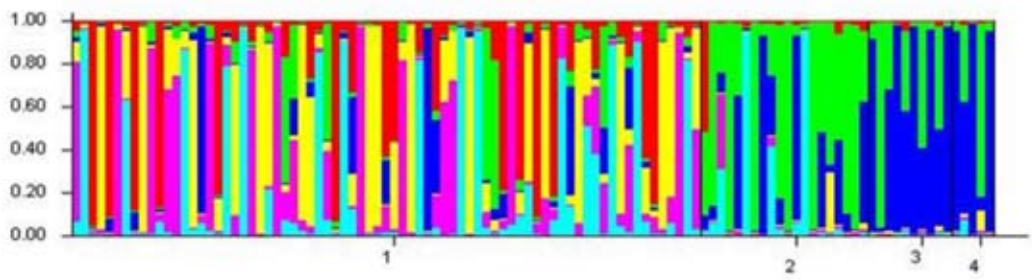

Fig 2. Graph separation of sampled populations of wild goat

low number of alleles is a sign of genetic bottleneck due to the isolation of populations or reduction of the effective population size. In our study all four populations from all loci showed deviation from the Hardy-Weinberg equilibrium. In all of the gene loci, a high heterozygosity deficit was observed. Many factors could explain these results including the Wahlund effect, null alleles, endogamy and optimum selection (Diz 2009). The findings of Microchecker software also confirmed the presence of null alleles in these gene loci. In this way, the main reason of heterozygosity deficit at the loci was the presence of null alleles. Xu *et al.* (2001) also report the presence of null alleles as the main reason of heterozygosity deficit.

Based on Wrihgt (1978) criterion, the F_{ST} values between 0.25-0.05 indicated high differentiation of samples. The differentiation index R_{ST} was 0.169. The values of F_{ST} and R_{ST} obtained from the molecular variance analysis were approximately equal. Slatkin (1995) and Roussot (1996) enumerated two reasons for the independence of differentiation from the mutation model: high immigration rate and short duration of population isolation. The AMOVA revealed that 83% of the variance had intra-population origin and only 17% of it was due to inter-population diversity. The high rate of intra-population diversity as compared to the inter-population diversity indicated that there was not any clear-cut genetic structure within the different populations (Diz, 2009). The low intra-population diversity and differentiation indexes reflect a high gene flow rate within the populations (Salehi, 2007). Our findings also showed a high gene flow rate in the studied loci (Tables 2 & 3). The resulted gene flow rate can be due to the natural migration between different regions. The report by Li et al. (2007) indicated that if Nm>1, then the gene flow should be enumerated as the main reason of genetic differentiation, while if Nm<1, it should be due to the genetic drift (Li et al. 2007). Therefore, our results suggested that the gene flow (Nm 1.061) was a major factor causing the genetic differentiation between the studied regions. Based on the assessment of data related to allele frequency, heterozygosity, Hardy-Weinberg Equilibrium, and F_{ST} index, it could be concluded that there were isolated populations of the wild goat in the regions with a low inter-population gene flow and high intra-population genetic diversity. Regarding the long distance between some of the regions, their high gene flow was justifiable using the Wahlund effect (Diz, 2009). As the Mentel's test indicated, there was an inverse relationship between the geographic distance of regions and the inter-population genetic exchange according to which the increase of geographic distance resulted in the decrease of genetic exchange rate (Freeland, 2005). Furthermore, the resulted phylogeny graph also did not show any isolation between the regions.

Table 5. Extent of genetic differentiation, gene flow, genetic distance

Genetic distance	Nm	Fst	Population 2	Population1
0.488	1.773	0.124	Muteh	Haftadqolleh
0.592	1.875	0.118	Jasb	Haftadqolleh
0.680	1.396	0.152	Rasvand	Haftadqolleh
0.322	1.688	0.129	Jasb	Muteh
0.696	1.845	0.228	Rasvand	Muteh
0.166	3.743	0.063	Rasvand	Jasb

Discussion

Based on our results, including low inter-population and high intra-population diversity, high genetic differentiation, closeness of F_{ST} index to R_{ST}, high gene flow, and non-isolated samples in the phylogeny graph, we concluded that the fragmentation of the populations of wild goat in Markazi Province had occurred for less than 30 years. Thus, the habitat fragmentation did not yet have any noticeable impact on the genetic diversity and structure of the wild goat population in Markazi Province. The protection of the extant population, inter-regional corridors, live capturing, relocating the wild goat from other regions to Jasb and Ravand, and restoring the wild goat in Alvand and Bazerjan in Tafresh, Iran, are among the appropriate solutions for preserving the genetic diversity of the wild goat population.

Table 6. Analysis of genetic bottleneck

Sign test			A sign rank test – Wilcoxon			Test Mode-Shift			Populations
IAM	SMM	TPM	IAM	SMM	TPM	IAM	SMM	TPM	
0.0138	0.0056	0.0302	0.0156	0.0156	0.0156	L-shaped distribution			Haftadqolleh
1.000	1.000	1.000	1.000	1.000	1.000	L-shaped distribution			Muteh
1.000	1.000	1.000	1.000	1.000	1.000	Non L-shaped distribution			Jasb
1.000	1.000	1.000	1.000	1.000	1.000	Non L-shaped distribution...			Rasvand

References

Adams J.R., B.T. Kelly, L.P. Waits 2003. Using faecal DNA sampling and GIS to monitor hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*). – Molecular Ecology, **12**: 2175-2186.

Ansari A. 2008. Monitoring Ecosystems and Natural Habitats of Markazi Province Identification of Degraded and Vulnerable Habitats . – J *DOE*, **47**: 22-32.

Askins R.A., M.J. Philbrick, D.S. Sugeno 1987. Relationship between the regional abundance of forest and the composition of forest bird communities. – *Biol. Conserv.*, **39**: 129-152.

Cornuet J.M., G. Luikart 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data . – *Genetics*, **144**: 2001-2014.

Diz P. A., P. Presa 2009. The genetic diversity pattern of *Mytilus galloprovincialis* in Galician Rias (NW Iberian estuaries). – *Aquaculture*, **287**: 278-285.

Eldridge W. H., K. Killebrew. 2008. Genetic diversity over multiple generations of supplementation: an example from Chinook salmon using microsatellite and demographic data. – *Conserv. Gen.*, **9**: 13-28.

ExtractMaster™ Fecal DNA Extraction Kit 2012. www.epicentre.com.

Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. – *Annual Review of Ecology Evolution and Systematics*, **34**: 487-515.

Freeland J. 2005. Molecular Ecology. Publications Jihad Mashhad University.

Khedrzadh C. 2010. Genomic DNA extracted from dung in order to devise appropriate protocols for genetic studies of wild and endangered species. In: Eleventh Iranian Genetics Congress, 1 to June 3 1389, Shahid Beheshti University, Tehran.

Li D., D. Kang, Q. Yin, Z. Sun, L. Liang 2007. Microsatellite DNA marker analysis of genetic diversity in wild common carp (*Cyprinus carpio* L.) populations. – *Genetics and Genomics*, **34**: 984-993

Malekian M. 2007. Effects of habitat fragmentation on the genetic diversity and population structure *Petaurus breviceps* species in southeast Australia. In: The National Biotechnology Congress of Iran 5-3 November.

Nei M. 1972. Genetic distance between populations. – *Am. Natur.*, **106**: 283-92.

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

Raymond M., F. Rousset 1995.: GENEPOP (VERSION 1.3) : Population genetic software for exact tests and ecumenicisim. – *J. Hered.*, **86**: 248-249.

Rousset F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation patterns. – *Genetics*, **142**: 1357-1362.

Shanjani Salehi P. 2007. Generations of genetic differentiation among populations of beech (*Fagus orientalis* Lipsky) Caspian forests. – *Iranian Journal of Biology*, **20**: 50-60.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. – *Genetics*, **139**: 457-462.

Valipor A. 2009. Whitefish Jewel of the Caspian Sea. – Fisheries Research Publications.

Van Oosterhout C. , W.F. Hutchinson, D.P.M. Wills, P. Shipley 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. – *Mol. Ecol. Notes*, **4**: 535-538.

Weinberg P., T. Jdeidi., M. Masseti., I. Nader., F. Cuzin 2008. *Capra aegagrus*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species .<http://www.iucnredlist.org/>.

Wright S. 1978. Evolution and the genetics of populations.Volume 4: Variability within and Among Natural Populations. University of Chicago Press,Chicago.

Xu Z., J.H. Primavera, L.D. De la Pena, P. Pettit, J. Belak, A.A. Warren 2001. Genetic diversity of wild and cultured black tiger shrimp (*Penaeus monodon*) in the Philippines using microsatellites. – *Aquaculture*, **199**: 13-40.

Ziaie H. 2008. A Fied Guide to the Mammals of Iran, Second *Edition*, 360-364.

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