Int. J. Environ. Res., 1(4): 321-324, Autumn 2007
ISSN: 1735-6865

Genetic study of Persian gazelle of Sohrein, Zanjan

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Received 15 May 2007; Revised 20 July 2007; Accepted 12 Aug. 2007

ABSTRACT: Persian gazelle (*Gazelle subgutturosa*) is one of the most important species in the world with its wide distribution in Iran The greatest number of this species in Iran is associated with the gazelles in Sohrain plain in Sorkhabad protected region in Zanjan province. Considering the significance of this species in the region and the fact that no genetic studies have been conducted to determine the status of this species, such genetic investigations were carried out in this study, 54 samples of the species hunted in 2005 were applied in this study. The samples were subjected for sequencing on D-LOOP region of the mtDNA. The results of this study indicated 17 polymorphism sites and 6 haplotypes in the region Haplotype type 1 and 5 with 72 and 1.85 percent presented the highest and lowest frequency in the population of gazelles. Based on the results obtained, the degree of variety of haplotypes in the population of gazelles of Sohrain region was estimated at 0.46 and nucleotide diversity was 0.84%. Based on the results of this study one can predict that population could be endangered owing to genetic depletion.

Key words: Mitochondrial DNA, Persion gazelle, haplotypes, Sohrein, Zanjan

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INTRODUCTION

About half of the species of plants and animals currently residing on the earth planet are expected to become extinct in less than a century. This drastic loss of biodiversity has been termed an extinction spasm and it is largely the result of anthropogenetic activity (Wright, 1977). Conservation genetics is both a theoretical and an applied science. Many population genetics and quantitative genetics theories have been proposed based on studies done on the populations of model organisms. Ideal model organisms, such as fruit flies and rodents, are inexpensive to maintain and reproduce very quickly in the laboratory. Conclusions obtained from experiments on these model organism populations can then be applied to endangered populations of animals found in captivity and in the wild (Hedrick, 2001).

One objective for conservation geneticists is to describe the genetic variation found in plants and animals. Genetic variation is critical to maintaining the biodiversity found in species, populations, and ecosystems and can be defined by allelic diversity and by the percent of heterozygosity found in a population. The percent heterozygosity in an individual or a population describes the number of genes or DNA regions found that have more than one allele. An allele is one of two or more different forms of a gene or DNA region. A population with many different alleles may be better capable of dealing with changes in its surroundings such as the introduction of a new disease. By definition, endangered organisms have small populations and therefore may have experienced a reduction in the level of genetic variation found in their populations (Conway, 2001). The objective method of this study was investigation of intraspecific genetic variation for (*Gazella subgutturosa*)

Sohrein, Zanjan region. This study is based on D-LOOP for prediction of genetic depletion of this population.

MATERIALS & METHODS

In 2005 a tissue sample was provided from the 54 gazelles in order to study DNA. After sampling out of their tissues, the samples were sent to Kiel University in Germany for genetic study purposes.

DNA extraction and purification

Total genomic DNA was isolated from the tissue of gazella. Standard procedures for isolation and purification of DNA were followed as described in Sambrook, (2002). The DNA was quantified using comparison between known concentrations of DNA and gazella genomic DNA by gel electrophoresis, and also using a spectrophotometer according to the manufacturer's instructions (Hofer Scientific). The DNA from tissue was stored at -20C for further analysis.

PCR condition

A PCR reaction was carried out on the gazella genome for obtained D-loop region in the mtDNA using designated primers. The 25 mL PCR reaction consisted of 10–15 ng of gazella tissue genomic DNA (template), 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM of dNTPs, 2 pmol of each primer, and 2.5 units of Taq polymerase (AB-gene). Cycling conditions were 5 min at 948˚C followed by 30 cycles of 1 min at 92˚C, 1 min at 92˚C, and 60 s at 58˚C, and finally followed by 10 min at 72˚C, using a Hybaid Omnigene Thermocycler. The PCR products were sequenced using automated sequencer ABI Prism 377.

As per the protocol QIAquik the centrifuge method was applied to purify and clean the PCR products. (Kit Protocol Company QIAquick, 2001). In brief: the products were obtained from the PCR after the investigation was made on the gel in order to determine the sequence by an automatic DNA-Sequencing so that haplotypes were specified by a study at the sequence in D-LOOP.

RESULTS & DISCUSSION

In this study 481 bp of D-LOOP region in the mtDNA were successfully isolated and sequenced. Vigorous analysis amount samples study in this region showed 17 polymorphism sites population, of which 11 of the mutations were transitions with 3.5% divergence while 6 were deletions/insertions (Table 1).

A point means, this position is identical to the first line.The number of the tissue samples extracted from 54 gazelles is shown in (Table 2) where haplotype type 1 and 5 with 72 and 1.85 percent presented the highest and lowest frequency in the population of gazelles. Haplotype diversity was 0.46 and nucleotide diversity was 0.84%. Data are indicatire of rare haplotypes.

As the above results show, the largest numbers of gazelles in the region of Sohrain, Zanjan, bear type 1 of haplotypes and 72 percent of these gazelles have the same haplotypes (Table 3). The mitochondrial DNA D-loop is hypervariable fragment sequence which is well known molecular marker in population genetic investigation (Hedrick, 2004). One case study showed that fragment sequence polymorphism was examined in 27 Mongolian gazelles from Mongolia, Russia, and China. Intraspecific polymorphism of the D-loop fragment examined was demonstrated. All haplotypes described were unique.

Table 1. Genetic variability of the D-LOOP obtained in using sequencing method

Sequences	23	125	142	148	156	157	158	159	160	161	285	310	320	324	329	341	393
1	T	A	G	G	*0	0	0	0	0	A	C	T	T	C	T	G	C
2	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	C	.	.	A	.	.	.	.	.	0	T	C	C	T	C	.	T
5	C	.	.	A	G	G	.	.	.	0	T	C	C	T	C	A	T
6	C	.	.	A	G	G	G	G	G	0	T	C	C	T	C	A	T

*0 is a gap

Table 2. Number of haplotypes collected from the Persian gazelle in the region of Sahrein, Zanjan

Gazelles	Haplotypes
39	1
1	2
1	3
7	4
1	5
5	6

Table 3. Percentage of haplotypes obtained in the population of Sohrein gazelles

Percent	Haplotypes
72.22	1
1.85	2
1.85	3
12.96	4
1.85	5
9.25	6

The average nucleotide diversity (pi) for the mtDNA fragment investigated constituted 5.85 +/ - 2.92% .A relatively high number of insertions and deletions was observed. In particular, a haplotype with the 77-bp insertion was described. The data obtained point to high genetic diversity of Mongolian populations. There was not any correlation between the distribution of haplotypes examined and geographical location of the animal tissue sampling sites (Sorokin, *et al*.,2005)**.** when we compared this result with Persian gazelles from Sohrein, Zanjan we find out that the average nucleotide diversity from Persian gazelles were 3.5% that it is very closed to Mongolian gazelles while 6 were deletions/insertions that shows low genetic diversity of Persian gazelles which is different with Mongolian gazelles. In the other study on the mitochondrial DNA (mtDNA) control region sequences from six Kenyan Grant's gazelle (*Gazella granti*) populations were highly divergent among locations. A similar level of divergence separates Grant's gazelles from a closely related species, the Soemmering's gazelle (*G. soemmeringii*). Nuclear microsatellite repeat number variation at two loci also indicated substantial population genetic differentiation. Despite high levels of sequence divergence populations of Grant's gazelles were more closely related to each other than to Soemmering's and Thompson's gazelles (G. thomsoni) as measured by nucleotide sequence divergence at the mtDNA protein coding cytochrome b gene and the nuclear alpha-lactalbumin gene. This pattern of extensive differentiation is hypothesized to have resulted from recently established contacts between formerly allopatric populations (Arctander, 1996). According to the this issue that the same Persian gazelles can be likely found in other parts of Iran the same investigation as that one experimented above can be conducted to determine whether Persian gazelles have suffered the phenomena (allopatric) similarly to the Grant gazelles or not. Investigating other areas of mt DNA and comparing its results with other data one can find out whether in other regions exist other low genetic varieties not. Meanwhile in a study on a species of the Arabic gazelle in Saudi Arabia Using 375 base pairs of mitochondrial DNA (mtDNA) cytochrome b gene derived from museum samples collected from the wild prior to the presumed extinction of this species, we show that *G. saudiya* is the sister taxon of the African dorcas gazelle (*G. dorcas*). Reciprocal monophyly of *G. saudiya* mtDNA haplotypes with *G. dorcas*, coupled with morphological distinctiveness, suggests that it is an evolutionarily significant unit. These data indicate that captive populations identified previously as potential sources of *G. saudiya*for captive breeding appear incorrectly designated and are irrelevant to the conservation of *G. saudiya*. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of several private collections of living gazelles in Saudi Arabia provides no evidence for the survival of *G. saudiya*. We recommend that field surveys be undertaken to establish whether *G. saudiya* is indeed extinct in the wild and that other private collections within the Arabian Peninsula be screened genetically. We urge caution when captive animals of unknown provenance are used to investigate the phylogenetics of cryptic species groups (Rebert, *et al*., 2001)**.**

CONCLUSION

Determining the present sequence in the area of controlling mt DNA reproduced by means of PCR technique reveal the low variety of haplotypes

and nucleotides in Sohrin gazelles. The haplotypes type 1, involves 72 percent of the gazelle populations in Sohrein, Zanjan. In other words, 72 percent of the population of this species bear a similar species of haplotypes. The results of the present study on the Persian gazelle from Sohrein, Zanjan show a low genetic variety in D-LOOP but it is too soon to judge whether this species has emptied with genetic features, or not. In addition to this information we suggest studies in the field of philogenetics. However the mtDNA data should be combined with ecological knowledge befor any suggestion.

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