

# Microsatellite Analyses of Genetic Diversity and Population Structure of Goitered Gazelle *Gazella subgutturosa* (Güldenstädt, 1780) (Artiodactyla: Bovidae) in Xinjiang, China

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**Abstract:** Goitered gazelle *Gazella subgutturosa* is an important ungulate animal inhabiting the desert and semi-desert areas from the Arabian Peninsula across the Middle East to Central Asia. Globally, the population size of goitred gazelle has dropped sharply due to the influence of natural factors and habitat fragmentation. To understand the genetic diversity and structure of goitred gazelle in Xinjiang, north-western China, we collected 153 samples from five geographic populations by using ten polymorphic microsatellite markers. The studied goitered gazelles exhibited low level of genetic diversity. The results of Bayesian clustering and Nei's unbiased genetic distance indicated the existence of some genetic differentiation among the specimens and the five geographic populations were divided into two groups. Bottleneck tests suggested that all five geographic populations have undergone a demographic bottleneck event. The present genetic information should be taken into account in management plans for the conservation of goitered gazelle in Xinjiang.

**Key words:** *Gazella subgutturosa*, microsatellite marker, genetic diversity, conservation management, Xinjiang

## Introduction

The goitered gazelle *Gazella subgutturosa* (Güldenstädt, 1780) (Artiodactyla: Bovidae) is a typical medium-sized animal inhabiting arid and semi-arid areas; it is widely distributed from Oman across the Arabian Peninsula to southern Turkey, following the steppes of Central Asia eastwards into central Mongolia and northern China (KINGSWOOD & BLANK 1996, MALLON & KINGSWOOD 2001). It is now locally extinct in Georgia, Kuwait, Armenia and Qatar, may be also in Yemen, Pakistan and Kyrgyzstan. It is almost at the border of extinction in Turkmenistan and in recent years, has drastically declined in many countries like Kazakhstan (DURMUŞ 2010, SOROKIN et al. 2011). This species has been classified as vulnerable in the Red Data Book of the International Union for Conservation of

Nature (IUCN) (IUCN Red List 2017) and has been ranked as one of the Class II key protected wild animals in China (WANG 1998). The wild populations of this species have declined dramatically in number over the past decades, owing to poaching, drought, extreme winter weather, livestock competition and habitat fragmentation (ZACHOS et al. 2010, DONG et al. 2016, IUCN Red List 2017).

In Xinjiang, the goitered gazelle can be seen in desert and desert steppe on the south-eastern margin of the Junggar Basin, western Tarim Basin, eastern Turpan Basin and Kumul Region. Previous studies on the ecology of goitered gazelle in Xinjiang concerned population size and density (CHU et al. 2009, EZIZJAN et al. 2015), social structure (QIAO et al. 2011) as well as assessment of habitat suit-

ability (RAHMUTULLA 2015, CHENG et al. 2016). Genetically, some conservation genetics and phylogenetic studies were conducted on the populations of goitered gazelle sampled from the Tianshan Wildlife Park (Lv et al. 2015), Kalamaili Ungulate Nature Reserve (DONG et al. 2016) and Ebinur Lake Wetland Nature Reserve (TAJIGUL et al. 2016) by using mtDNA sequences. A moderate haplotype diversity and lower nucleotide diversity in wild goitered gazelle throughout their natural range in Xinjiang was revealed using mitochondrial DNA control region (SHAMSHIDIN et al. 2018a) and cytochrome *b* gene (SHAMSHIDIN et al. 2018b), whereas high genetic diversity was calculated in samples from Kumul, Qarqan and Hejing in Xinjiang by using ten pairs of microsatellite markers (BUZOHRA et al. 2016). Nonetheless, in these studies, the population genetic information was limited because of inadequacy of sample sources or being confined to mitochondrial markers.

To utilise the wild genetic resources of goitered gazelle effectively and to provide a sustainable management plan for this species in Xinjiang, it is also important to carry out a comprehensive study on its genetic diversity and to acknowledge that the genetic structure and differentiation of populations has crucial conservation implications. Among the many existing genetic markers, the microsatellite is one of the best-suited markers to deal with such problems (GOLDSTEIN et al. 1997). In this paper, the genetic diversity and structure of goitered gazelle was examined by studying wild individual specimens sampled from its natural distribution areas in Xinjiang using ten polymorphic microsatellites.

## Materials and Methods

### Sample collection and DNA extraction

A total of 153 specimens of goitered gazelle from five geographic populations in Xinjiang, northwestern China, were used in this study: 65 from Junggar Basin (JU), 12 from Yiwu (YW), 12 from Kumul (KU), 45 from eastern Tarim Basin (ET) and 18 from western Tarim Basin (WT), from October 2013 to November 2014 (Fig. 1, Table 1). Fresh faecal samples and pieces of muscle were collected and preserved in the sterile tubes with anhydrous alcohol at  $-20^{\circ}\text{C}$  until DNA extraction. The total genomic DNA was extracted from faecal samples using and improved CTAB lysis method (RISALAT et al. 2012) and from muscle samples following the standard method of proteinase-K digestion, phenol chloroform extraction and precipitation in ethanol. The concentration and purity of the genomic DNA were

detected by 1.0% agarose gel electrophoresis. The extracted DNA in 30  $\mu\text{L}$  of TE buffer was preserved at  $-80^{\circ}\text{C}$  until used.

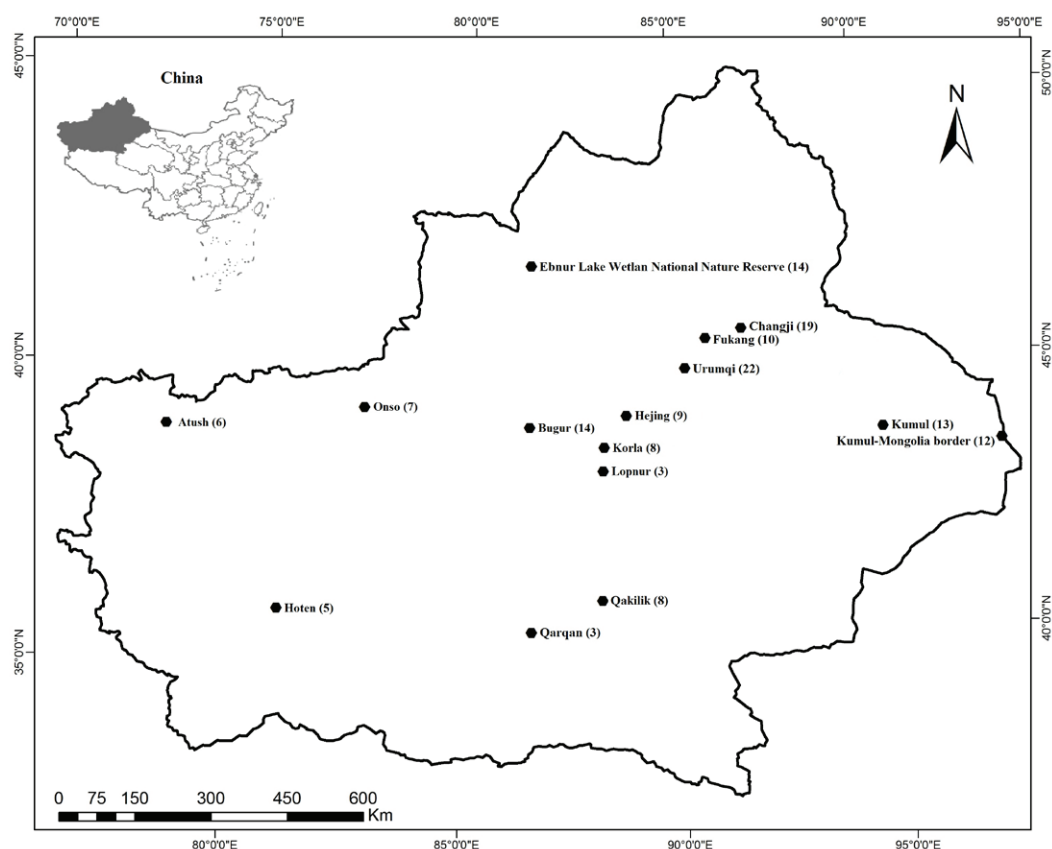
### Microsatellite loci amplification and genotyping

Ten pairs of microsatellite primers (BM888, BM4208, BM4513, 0arFCB304, BM203, SR-CRSP9, 33HDZ496, 33HDZ8, OraFCB005, BM4621) were chosen based on their high polymorphism as determined in our previous work (NING et al. 2016). The forward primer of each locus was fluorescently labelled with FAM, TAMRA or HEX at the 5' end (Table 2). Primers were synthesised by Sangon Biotech (Shanghai, China) and dissolved in deionised water. PCR was performed in a 20  $\mu\text{L}$  reaction volume: 2  $\mu\text{L}$  10 $\times$ Buffer (containing  $\text{Mg}^{2+}$ ), 1  $\mu\text{L}$  of dNTPs, 1  $\mu\text{L}$  each primer (10  $\mu\text{M}$ ), 2  $\mu\text{L}$  of genomic DNA, 1.5  $\mu\text{L}$  of bovine serum albumin (20  $\mu\text{g}/\mu\text{L}$ ), 0.2  $\mu\text{L}$  Primer Star Max DNA Polymerase and ddH<sub>2</sub>O to make up the total volume to 20  $\mu\text{L}$ . The PCR amplification was conducted as follows: initial denaturation at  $95^{\circ}\text{C}$  for 5 min, followed by 33–35 cycles of denaturation at  $95^{\circ}\text{C}$  for 45s, annealing at specific temperature (Table 2) for 60s, extension at  $72^{\circ}\text{C}$  for 60s and a final extension at  $72^{\circ}\text{C}$  for 10 min. PCR products were stored at  $4^{\circ}\text{C}$  and 3  $\mu\text{L}$  of the amplification product was visualised on 1.5% agarose gel after staining with GelRed™. Successfully amplified PCR products were detected by ABI 3730XL capillary sequencer (Applied Biosystems) with LIZ® 500-bp as internal size standard (GODINHO et al. 2012). The microsatellite allele sizes were independently scored and binned using GeneMapper software version 3.2.

### Data analyses

General estimates of genetic diversity including the number of alleles ( $N_a$ ), number of effective alleles ( $N_e$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), genetic differentiation coefficient ( $F_{IS}$ ) and deviation from Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP version 1.2 (RAYMOND & ROUSSET 1995). The polymorphic information content (PIC), power of discrimination (DP) and cumulate DP (TDP) were estimated using CERVUS version 3.0 (KALINOWSKI et al. 2010).

To estimate the genetic structure of populations, we used the Bayesian individual assignment using STRUCTURE version 2.3.4 (PRITCHARD et al. 2000). For each K, we run ten times with 105 MCMC steps after an initial burn-in of 104 iterations; to assess convergence of Ln Pr (X|K) for K from 2 to 15, the number of clusters present was then determined by an *ad hoc* statistic  $\Delta K$  (EVANNO et al. 2005). The



**Fig 1.** Map of the sampling sites of goitered gazelle populations in Xinjiang, China, showing the sample size at each locality (in parentheses).

**Table 1.** Location of populations and number of individuals sampled in this study.

Population	Sampling site	No. of samples	Longitude	Latitude	Sample type	No. of individuals
Junggar Basin population	Ebnur Lake Wetland National Nature Reserve	14	83°12'15"	44°38'55"	Faeces	12
	Changji	19	88°49'33"	44°28'33"	Faeces	15
	Fukang Santai Reservoir	10	87°59'04"	44°09'21"	Faeces	10
	Urumqi Tuoli Village	22	87°38'40"	43°31'56"	Faeces	22
Yiwu	Kumul-Mongolia border in Yiwu County	12	95°50'32"	43°17'01"	Faeces	12
Kumul	Liu Shu Quan in Kumul	13	92°50'19"	43°11'14"	Faeces	12
Eastern Tarim Basin population	Hejing	9	86°29'19"	42°25'32"	Faeces	7
	Bugur	14	84°14'57"	41°46'35"	Faeces	13
	Korla	8	86°09'10"	41°45'52"	Faeces	5
	Lopnur	3	86°15'40"	41°20'13"	Muscles	3
	Qakilik	8	86°58'31"	39°01'08"	Faeces, muscles	6
Western Tarim Basin population	Qarqan	3	85°31'38"	38°08'45"	Muscles	3
	Mouyu County in Hoten	5	79°44'09"	37°16'35"	Muscles	5
	Wu Qia County in Atush	6	75°51'35"	39°47'52"	Faeces	5
	Onso	7	80°14'12"	41°16'33"	Faeces	6
	Total	153				136

analysis of bottleneck events was done using the Wilcoxon sign-rank test with BOTTLENECK version 1.2.02 (CORNUET & LUIKART. 1996). It was used to estimate the probable influence of demographic changes on genetic diversity, supposing mutations of microsatellite loci under different mutation mod-

els such as infinite allele model-IAM, strict stepwise mutation model-SMM (OHTA & KIMURA 1973) as well as the intermediate two-phase model-TPM (DI RIENZO et al. 1994). Nei's unbiased genetic distance and Nei's genetic identity (NEI 1978) among populations were calculated using GENEPOP version 1.2

**Table 2.** Characterisation and individual identification of ten microsatellite loci used for the study. Locus, primer sequences, fluorescently labelling, annealing temperature (Ta, °C), number of alleles found (Na), expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), polymorphism information content (PIC), power of discrimination (DP), cumulative power of discrimination (TDP).

Locus	Primer sequence (5'-3')	Fluorescently labelling	Ta	Na	$H_e$	$H_o$	PIC	DP	TDP
OarFCB-304	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	5' HEX	55	9	0.598	0.174	0.509	0.737	0.9762
BM4208	TCAGTACACTGGCCACCATG CACTGCATGCTTTTCCAAAC	5' FAM	57	7	0.596	0.297	0.496	0.693	
SR-CRSP9	CTAGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	5' HEX	50.5	9	0.455	0.341	0.392	0.529	
BM4513	GCGCAAGTTTCCTCATGC TCAGCAATTCAGTACATCACCC	5' TAMRA	50	9	0.695	0.495	0.605	0.796	
33HDZ496	GTTTTTCCAGATGGTATTTTCTC GTATTCGGCTGAAGGGACC	5' FAM	54.5	6	0.562	0.585	0.462	0.770	
33HDZ8	F- G ACAAACACTCAGAAGGCAAAG R- GGTGGCAGGACTGAGCAAG	5' FAM	52.5	9	0.773	0.583	0.684	0.845	
OarFCB-005	AAGTTAATTTCTGGCTGGAAAACCC GACCTGACCCTTACTCTCTTCACTC	5' TAMRA	50	9	0.780	0.799	0.697	0.824	
BM203	GGGTGTGACATTTTGTT CCC CTGCTGCCACTAGTCCT TC	5' HEX	50	8	0.244	0.111	0.202	0.629	
BM4621	CAAATTGACTTATCCTTGGCTG TCTAACATATGGGCTGCATC	5' TAMRA	58	4	0.337	0.416	0.274	0.571	
BM888	AGGCCATATAGGAGGCAAGCTT CTCGGTGAGCTCAAAAACGAG	5' HEX	58	4	0.417	0.049	0.335	0.575	
Mean				7.4	0.5458	0.3849	0.4655		

(RAYMOND & ROUSSET 1995). In order to visualise the relationship between the five geographic populations, an UPGMA tree was built with the software MEGA 4.1 (TAMURA et al. 2007). The correlation between Nei's genetic distance and geographic distance (km) was estimated using the Mantel test in GenALEX 6.5 (PEAKALL & SMOUSE 2012). Statistical significance was evaluated by performing 9999 permutations. Nei's genetic distance among populations were calculated using GENEPOP version 1.2 (RAYMOND & ROUSSET 1995) and geographic distances were calculated on the basis of sample locations for the average geographic coordinates of each population by using the ArcGIS v.9.3.

## Results

### DNA extraction and individual identification

In this study, a total number of 153 genomic DNA was successfully extracted from 138 fresh faecal samples and 15 muscle samples. Ten microsatellite markers were successfully amplified in five geographic populations, the number of alleles per locus ranged from 4 to 9 (Table 2) and a total of 74 different alleles were detected. The PIC value in different loci ranged from 0.202 (BM203) to 0.697 (OarFCB-005), the average PIC was 0.4655 (Table 2). The power of discrimination of the ten microsatellite loci was 0.529 to 0.845

and the cumulative individual recognition rate was 0.9762 (Table 2). Final individual identification determined that the 138 faecal samples belonged to 121 different individuals, and the rate of sample repetition in this study was 12.31%.

### Genetic variability and Hardy-Weinberg Equilibrium (HWE)

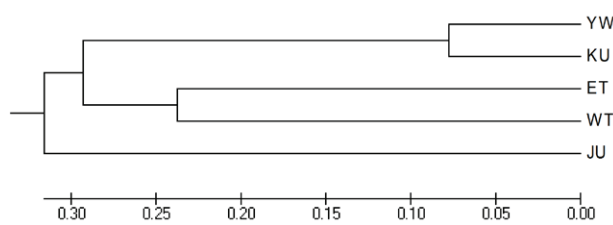
The average number of alleles per population ranged from 2.63 (WT) to 4.75 (JU, Table 3). The average  $H_o$  and  $H_e$  was 0.3849 and 0.5458, respectively. The highest genetic diversity was observed in the JU population ( $PIC=0.539$ ,  $H_e=0.599$ ) and the lowest in WT population ( $PIC=0.392$ ,  $H_e=0.502$ ). The F-statistics showed that positive  $F_{IS}$  values were found in every population (Table 3). The values ranged from 0.198 (ET) to 0.425 (KU) and the mean was 0.286. The HWE tests indicated that all populations deviated significantly from HWE ( $P<0.05$ ) at most microsatellite loci. The JU, YW, KU, ET and WT populations had 10, 4, 6, 7 and 4 microsatellite loci departing from HWE, respectively (Table 3).

### Population structure and bottleneck detection

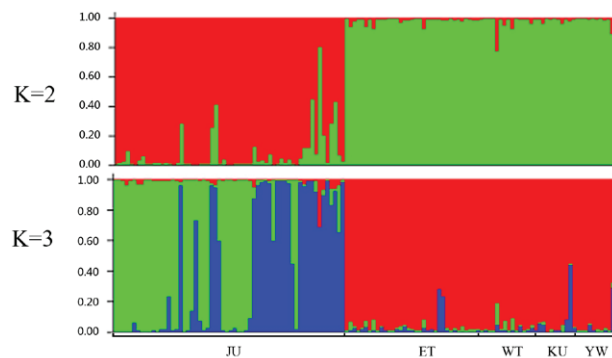
**The genetic relationships among the five populations** were depicted with a dendrogram based on the Nei's genetic distance (Fig. 2). The populations were differentiated into two main clusters in which

**Table 3.** Summary statistics of genetic variation per population. Number of samples ( $n$ ), number of alleles per locus ( $N_a$ ), effective number of alleles per locus ( $N_e$ ), polymorphism information content ( $PIC$ ), expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), Shannon's information index ( $I$ ), F-statistics of genetic differentiation coefficient ( $F_{IS}$ ) and number of loci with significant departures from Hardy–Weinberg- equilibrium (No HWE,  $P_{HW} < 0.01$ ) are given for each population. Abbreviations of the population are given in the paper.

Populations	n	$N_a$	$N_e$	$PIC$	$H_o$	$H_e$	$I$	$F_{IS}$	No HWE
JU	59	4.7500	2.8263	0.5399	0.4505	0.5987	1.1449	0.276	10 (all loci)
YW	12	3.8000	2.8663	0.4585	0.3917	0.5268	0.9656	0.234	4 (OarFCB-304, BM4513, 33HDZ496, BM888)
KU	12	4.3000	3.2337	0.5321	0.3333	0.5931	1.1217	0.425	6 (OarFCB-304, BM4208, SR-CRSP9, BM4513, 33HDZ8, BM888)
ET	37	3.0167	2.3164	0.4050	0.3899	0.5083	0.8105	0.198	7 (OarFCB-304, BM4208, SR-CRSP9, 33HDZ496, 33HDZ8, BM203, BM888)
WT	16	2.6333	2.1213	0.3922	0.3589	0.5022	0.7566	0.296	4 (OarFCB-304, BM4208, BM203, BM888)
Mean	27.2	3.7000	2.5809	0.4655	0.3849	0.5458	0.9598	0.286	



**Fig 2.** UPGMA dendrogram of five geographic populations of goitered gazelle in Xinjiang based on Nei's genetic distance at ten microsatellite loci.



**Fig 3.** Assignment of individuals on the basis of STRUCTURE analysis on the microsatellite allele data. Detecting the most likely number of genetically distinct groups within the goitered gazelles in Xinjiang based on percentage of population assignments. The sampling populations for individuals are shown as JU (Junggar Basin population), ET (Eastern Tarim Basin population), WT (Western Tarim Basin population), KU (Kumul population) and YW (Yiwu population).

YW and KU populations clustered with ET and WT populations, JU population formed a separate branch and the two branches then clustered into one dendrogram. Our Bayesian clustering analysis provided additional insights into the genetic structure of goitered gazelle in Xinjiang. The assignment test analysis for all 136 goitered gazelle samples conducted

**Table 4.** Bottleneck detection within *Gazella subgutturosa* samples assuming different mutation models. The  $P$  values represent probabilities of heterozygosity excess under different mutation models obtained with the Wilcoxon unilateral testing.

Populations	Number of heterozygosity excess loci			P value		
	IAM	TPM	SMM	IAM	TPM	SMM
Group 1	7	4	2	0.34485	0.17209	0.01465
Group 2	5	4	1	0.4	0.1726	0.00192

in STRUCTURE showed the most likely partitioning of the genetic structure was obtained with  $K=2$  (Fig. 3). The goitered gazelle populations studied were divided into two groups.

The results of Wilcoxon sign-rank test analysis including the groups defined by STRUCTURE analysis are shown in Table 4. The sign test IAM, TPM and SMM represented excesses in 7, 4 and 2 loci in the group one populations; 5, 4 and 1 loci in the group two populations. No statistically significant gene diversity excess was observed under mutation models IAM and TPM in both groups but assuming the mutation model SMM, significant heterozygosity excess was detected in the two groups. Our results suggested a most probable recent genetic bottleneck in the population of goitered gazelle in Xinjiang.

## Discussion

### Genetic diversity

Genetic diversity is one of the most important attributes of any population and loss of genetic diversity has direct disadvantageous effects on species' survivability (DAVID 1998), making the species vulnerable to extinction (HEDRICK et al. 1995). The comprehensive understanding of genetic variability

is of great significance for the protection of endangered animals (FREELAND 2011). *PIC* is a method for measurement of genetic diversity: the greater the polymorphism information content, the greater the proportion of heterozygote in the population and the higher the genetic information is provided. In this work, we strive to increase the amount of informative markers for populations of goitered gazelle in Xinjiang. For this purpose, ten microsatellite loci have been chosen following BUZOHRRA et al. (2016). The average *PIC* value of the ten microsatellite loci used in this study was approximately 0.4655, following the criteria of BOTSTEIN et al. (1980). Only one locus (BM203) belonged to a low polymorphic site, four loci (OarFCB-304, BM4513, 33HDZ8, OarFCB-005) belonged to highly polymorphic sites and the others – to moderately polymorphic sites.

Previous studies (TING et al. 2009, ZACHOS et al. 2010) revealed that the HWE deviation is possibly caused by inbreeding and genetic drift. Inbreeding may increase the proportion of homozygous individuals within a population, which ultimately leads to a reduction in fitness (CRNOKRAK & ROFF 1999). In the present study, each locus except for OarFCB-005 and BM4621 in different sampling sites, was significantly deviated from HWE ( $P < 0.01$ ). These sites exhibit lower number of heterozygotes than expected and most of the HWE deviations were related to the high positive inbreeding coefficient. We found that all populations exhibited positive  $F_{IS}$ . Therefore, we could assume that the departure from HWE was probably caused by either random sampling from different populations or an excess of homozygotes (or heterozygote deficiency) for single loci and that was most likely due to inbreeding (PEMBERTON et al. 1995, TING et al. 2009).

In our study,  $H_e$  was higher than  $H_o$  in all populations. KHOSRAVI et al. (2017) reported that the average value of  $H_e$  for *Gazella subgutturosa* in central Iran was 0.540. The mean  $H_e$  value of *G. dorcas* populations in North-Western Africa was 0.564 (GODINHO et al. 2012) and for *Gazella granti* in Kenya was 0.590 (HUEBINGER et al. 2006). However, the mean  $H_e$  value (0.546) of goitered gazelle population in this study was more similar to that of previously reported for same species or for related gazelle species but much lower than wild *Gazella subgutturosa* in the north-west of Iran ( $H_e = 0.72$ , ZACHOS et al. 2010), *Gazella granti* in Kenya ( $H_e = 0.61$ , ARCTANDER et al. 1996) and than the Mongolian gazelle ( $H_e = 0.72$ , OKADA et al. 2015). Meanwhile, the genetic diversity of all populations ( $H_e = 0.546$ ,  $PIC = 0.466$ ) observed in this study was lower than the one calculated for

the populations from Kumul, Qarqan and Hejing in Xinjiang through using ten microsatellite loci ( $H_e = 0.756$ ,  $PIC = 0.715$ , BUZOHRRA et al. 2016). The difference in genetic diversity may be attributed to the different types of microsatellite markers used as well as to the different sampling sites that we have studied. We estimated low level of genetic diversity of goitered gazelle populations in Xinjiang. Several factors may have contributed to this observation. Firstly, null alleles could lead to increase in the number of homozygotes (Lv et al. 2014). Secondly, inbreeding as reflected by deviations from HWE in all populations detected in this study. Finally, limited genetic exchange among populations probably caused by natural obstacles such as the Tianshan Mountains (GUZALNUR 2015). Another reason for the low genetic variation was that some of the loci (BM203, BM888 and BM4621) that we had chosen might not reflect the real genetic diversity of goitered gazelle populations sampled in this study.

The genetic diversity in the KU and WT populations were lower as compared to the other three populations, indicating the significant decrease in their heterozygosity; the low degree of heterozygosity might be explained by the recent bottleneck event and inbreeding. The Taklamakan Desert in the central Tarim Basin forms a barrier for genetic communication of WT population with the other populations especially with JU, YW and KU populations. That may intensify the mating of close relatives and remarkably reduce the genetic diversity of WT population. The considerably fragmented distribution of WT population was previously reported (RAHMUTULLA 2015) and might be the main factor decreasing its genetic diversity.

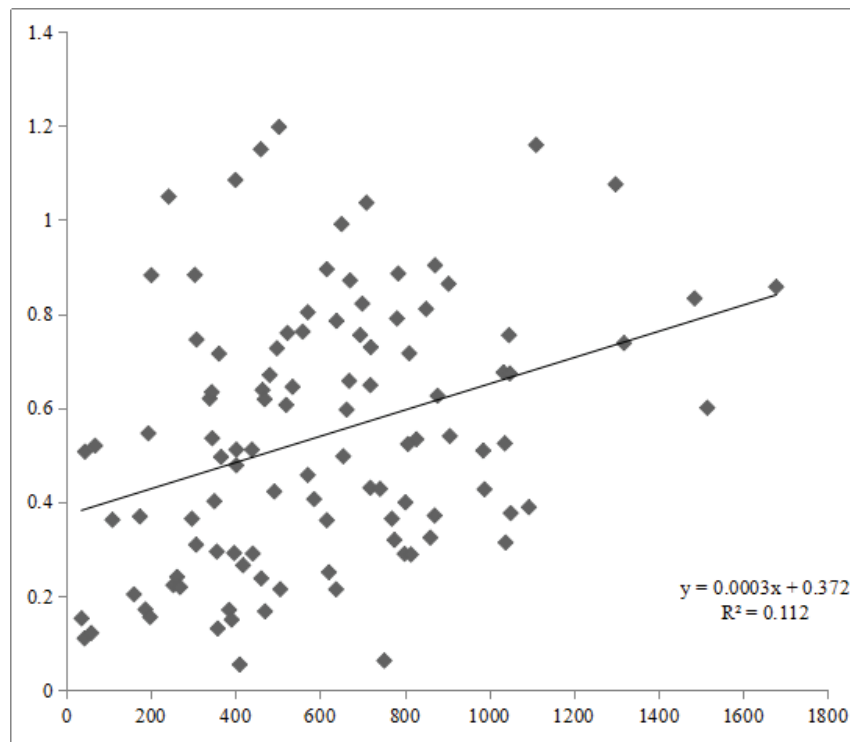
It is important to identify populations that have experienced a bottleneck due to the severe reduction in population size and the associated loss of genetic variation and fixation of deleterious alleles (MAUDET et al. 2002). Bottleneck events decrease the desired level of heterozygosity of the population and increase the rate of inbreeding (FREELAND 2011). In our study, the goitered gazelle populations in Xinjiang showed signs of a recent bottleneck based on the SMM results, that indicate a low effective population sizes for each population (TING et al. 2009). The recent bottleneck events may be another reason for the reduced genetic diversity of the studied populations and these results are also congruent with previous studies (SHAMSHIDIN et al. 2018b). It was reported that, this population has gone through the process of prosperity and decline in recent decades (QI et al. 2011). At the beginning of last century there were as many as 40,000 or 500,000 goitered

gazelle in Xinjiang (FENG 2003). However, in the last century the population number of this species decreased dramatically due to natural disasters and poaching (QIAN 2001). In addition, the massive destruction of natural vegetation and degradation of ecosystems (QIAN 2001), livestock competition (XU et al. 2008), extreme winter weather (DONG et al. 2016), as well as habitat fragmentation caused by agricultural land expansion and desertification (SUN et al. 2002, RAHMUTULLA 2015) may be the main reasons for reduction in population size (bottleneck) of goitered gazelle in the past decades.

The goitered gazelle in China has been under legal protection since 1980 (JIANG & SUNG 2001) and has been listed as Category II protected animals in 1989 (WANG 1998). In recent years, due to the strengthening of conservation and management, the average density of *G. s. sairensis* in Junggar Basin has increased from  $0.71 \pm 0.17$  individual/km<sup>2</sup> in the early 1990s to  $0.8 \pm 0.2$  individual/km<sup>2</sup> in the mid-1990s (GAO et al. 1996). It was estimated to be about 13,000-24,000 individuals in the Changji Prefecture and 7,000-18,000 in the Kalamaili Ungulate Nature Reserve; around 5580 individuals were reported elsewhere in Xinjiang (SU et al. 1999) This number is much smaller than the recommended population size needed to retain the species evolutionary potential (FRANKHAM et al. 2002).

**Genetic structure**

The STRUCTURE analysis for all 136 goitered gazelle samples found that the highest value for the estimated log-likelihood (Ln Pr(X/K) or Ln P(D)) was obtained at K= 9, while for K > 2, the LnP (D) values indicated only a slight increase. According the *ad-hoc* statistic  $\Delta K$ , the most likely partitioning of the genetic structure was obtained with K = 2. The Bayesian clustering analysis also proved that only at K = 2 the geographic boundary among the two clusters was clear and most individuals were not assigned to any group with K =3 (Fig. 3). The structure analysis showed that the populations of the goitered gazelle in Xinjiang were divided into two groups, this was consistent with the results of mtDNA control region (SHAMSHIDIN et al. 2018a). Some level of genetic differentiation existed among the five geographic populations. Study of goitered gazelle in Central Iran (KHOSRAVI et al. 2017) revealed that high mountains, roads, warm and dry climates, as well as poor vegetation cover restrict the dispersal of this species and affect the population structure. These factors are highly similar to the living conditions of the goitered gazelle in Xinjiang (QIAO et al. 2011). RAHMUTULLA (2015) showed that the considerably fragmented distribution of goitered gazelle populations in Xinjiang, especially in WT population, might have a potential effect on dispersal



**Fig 4.** Mantel test for matrix correlation between Nei's genetic distance and geographic distance for goitered gazelle populations in Xinjiang, China

that could be responsible for the genetic differentiation among populations of this species.

The Mantel test revealed a very weak but positive correlation between Nei's genetic distance and geographic distance (km) ( $R^2=0.1$ ,  $P=0.03$ ; Fig. 4). We could speculate that the genetic differentiation between the five geographic populations might be caused by geographic isolation due to distance, which may be playing an important role in genetic exchange among populations. Apparently, the very weak positive correlation between the Nei's genetic distance and the geographic distance indicates that there are other factors (i.e. highway and railway construction, landscape features) possibly affecting the genetic distances (KHOSRAVI et al. 2017).

In this study, the greatest genetic distance was found between WT and KU populations (0.9099) and this value was much higher than that of between ET and KU populations (0.5145), mainly owing to the high gene flow between the ET and KU populations (SHAMSHIDIN et al. 2018a). Another possible explanation of the genetic distance in this study could be partially due to the Tianshan Mountains, Gurban-tunggut Sandy Desert and the Taklamakan Desert. These natural obstacles block the gene exchange between JU and the other four populations. Interestingly, we recorded the highest genetic similarity between YW and KU populations despite the fact that the Tianshan Mountains separates them. These conclusions coincide with the results of RAHMUTULLA (2015), which suggest that the habitat of the two populations is continuous and without fragmentation. DONG et al. (2016) have shown that there is a channel from Mulei Large Stone areas in the east of the Tianshan Mountains to the Kumul Qiangjing areas, through which the goitered gazelle on the south slope of the Tianshan Mountains are able to move from north to south at the right time. Our results further suggest that the high genetic exchange between the YW and KU populations has probably been formed through this channel.

In summary, the genetic structure of the goitered gazelle observed in this study can be explained by habitat distribution, climatic conditions, natural obstacle avoidance and highway and railway construction.

### Implications for conservation management

Conservation genetics can provide information contributing to conservation planning; analysing the genetic diversity and the genetic structure of populations are important to provide a theoretical basis for planning future conservation strategies (MOCKFORD et al. 2005). Researches indicate that the genetic

variability is believed to be the key to allow natural populations to adapt to their changing environment and low genetic diversity in population can lead to a decline in viability, especially under the influence of adverse environmental conditions (COLTMAN et al. 1999, KRISTENSEN et al. 2005).

Therefore, it is very important to take effective conservation priorities for increasing population size and genetic exchange among populations and to avoid inbreeding within populations (TING et al. 2009). To achieve that, we propose some conservation methods for goitered gazelle in Xinjiang: (1) considering the population genetic structures observed, the population of goitered gazelle in Xinjiang should be protected as two basic protection units; (2) special conservation consideration should be given to the WT and KU populations, which exhibited the lowest genetic diversity; (3) potential effects of road networks on dispersal of goitered gazelle should be taken into account when designing local conservation programs: it is crucial for the establishment of mitigation measures such as wildlife crosswalk when projecting the construction of new highways.

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