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RESEARCH ARTICLE

Genetic diversity, population genetic structure and demographic history of Przewalski's gazelle (*Procapra przewalskii*): implications for conservation

Ji Yang · Zhigang Jiang

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Abstract The Przewalski's gazelle (*Procapra przewalskii*) is one of the most endangered antelope species in the world. It is endemic to China and is a flagship species in the eastern part of the Qinghai–Tibet plateau. To establish effective conservation measures on this species, genetic information such as genetic structure is needed. However, there has not been a comprehensive genetic assessment on this gazelle using nuclear DNA markers yet. Here, we employed 13 microsatellite loci to investigate genetic diversity, population genetic structure and demographic history of Przewalski's gazelle using noninvasive samples of 169 wild gazelles collected from nine populations. A total of 76 alleles were detected from the entire samples, mean allele number was 5.85, and overall H_O and H_E were 0.525 and 0.552, respectively. Structure and GENELAND analyses found six genetic groups in the nine populations. Between the inferred genetic groups, significant genetic differentiation and low migration rates were detected. Demographic analyses indicated that Przewalski's gazelle experienced genetic bottleneck and severe population decline, with the ancestral effective population size reducing to less than one percent. Based on the results of this study, we provide several conservation recommendations for Przewalski's gazelle, such as six management units, periodic monitoring and special conservation consideration on the Qiejitan population.

J. Yang · Z. Jiang
Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, No. 1-5 Beichen West Road, Beijing 100101, China

J. Yang · Z. Jiang (✉)
Graduate School of the Chinese Academy of Sciences, No. 19 (A) Yuquan Road, Beijing 100049, China
e-mail: jiangzg@ioz.ac.cn

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Introduction

In conservation biology, genetic considerations such as genetic diversity, population genetic structure and demographic history are increasingly needed for either immediate or long-term conservation planning (Frankham et al. 2002). Genetic diversity is the fundamental element of biodiversity, it is crucial for the evolutionary potential of endangered species (Frankel 1974; Frankham 2005). Population genetic structure is the distribution pattern of genetic diversity, it indicates current genetic relationships among populations. Demographic history provides the information of genetic bottleneck and population size change. Thus they are important for establishing effective conservation measures, such as the identification of appropriate management unit (MU) for endangered species (Moritz 1994).

The Przewalski's gazelle (*Procapra przewalskii*) is considered to be one of the most endangered antelope species in the world (Mallon and Kingswood 2001). It is endemic to China and is a flagship species in the eastern part of the Qinghai–Tibet plateau (Jiang et al. 2001). The gazelle once inhabited large area of Gansu, Inner Mongolia, Ningxia and Qinghai Provinces, however, it has experienced sharp population declines, now only a few hundred individuals survive in several isolated locations around the Qinghai Lake (Jiang et al. 1995, 2000; Jiang 2004; Ye et al. 2006). Accordingly, it is listed as 'Endangered' under the IUCN Red List (2008). It is also listed as a Category I

species under the Wild Animal Protection Law of China and regarded as a conservation priority species together with other 14 endangered species in China (Jiang et al. 2003).

Studies have been carried out on Przewalski's gazelle since 1990s, including group pattern, food competition, population viability, sexual behavior, phylogeny, phylogeography et al. (Lei et al. 2001, 2003a, b; Li and Jiang 2002; Liu and Jiang 2004; You and Jiang 2005). In genetic context, Lei et al. (2003b) clarified that Przewalski's gazelle is an independent species through mitochondrial DNA (mtDNA) 12S and 16S RNA sequences; they also found low nucleotide diversity and significant genetic structure in the four populations known at that time using mtDNA control region sequences (Lei et al. 2003a). Recently, more populations were discovered, but there has not been a new genetic assessment on whole species. Further, previous intraspecific genetic assessment was only based on matrilineal mtDNA marker which can not reflect the genetic status in both sexes. Hence, there is an urgent need to carry out a comprehensive genetic assessment on all known populations using nuclear DNA markers inherited from both parents.

In this study, we used 13 nuclear microsatellite loci to solve the following questions: (1) what level of genetic diversity in Przewalski's gazelle currently? (2) Is there population genetic structure within this gazelle on the basis of nuclear genotype data? (3) Did the gazelle experience genetic bottleneck or population size change in the past? Based on the results of this study, we discussed current genetic status of Przewalski's gazelle and provided several conservation recommendations such as MUs for this endangered species.

Materials and methods

Study area and sample collection

The study area (98°26′–100°54′ E, 36°13′–37°36′ N) was around the Qinghai Lake. Qinghai Lake is the largest inland saline lake in China, and it is a perennial lake which freezes in winter. The lake has an area of 4300 km^2 and an average water depth of 16 m (max. 28 m). The elevation in the study area ranges from 2,900 to 3,800 m above sea level, and the climate is cold and dry (Jiang et al. 2000). Main vegetation types include alpine meadow, shrub vegetation and psammophilous vegetation (Jiang et al. 2000). Samples were collected from all known populations (Fig. 1) including Yuanzhe (P1), Hudong (P2), Ketu (P3), Shadao (P4), Ganzihe (P5), HerG (P6), Bird Island (P7), Shengge (P8), and Qiejitan (P9). Population P9 is located in the Gonghe Basin, and other populations are located in the Qinghai Lake Basin which is surrounded by several mountains (Fig. 1). We collected skin samples from wolf-killed gazelles from 2004 to 2007 and stored them at –20°C. We collected fresh fecal samples in winter (Nov–Dec 2006), because it has been demonstrated that wild

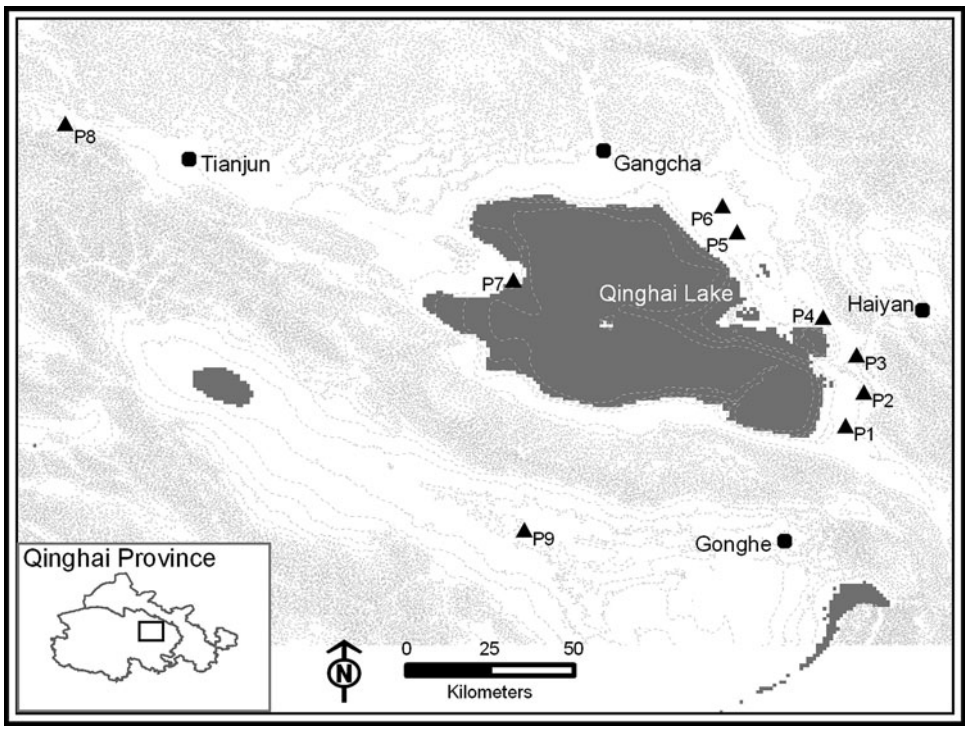

Fig. 1 Map of study area. The location of nine sampling populations (P1–P9) in Przewalski's gazelle is represented as *black triangle*. *Black square*, *dark grey* area and *dotted line* indicates county capital, lake and contour line respectively. The six genetic groups inferred in this study are: G1 = P1; G2 = P2, P3, P4; G3 = P5, P6; G4 = P7; G5 = P8; G6 = P9

ungulate faeces sampled in winter had low genotyping error rates (Maudet et al. 2004). The fecal samples were preserved in 100% ethanol. In each population, after a group of gazelles was located, we approached the gazelles and collected all fresh feces from the ground. Due to very small population sizes in P4 and P7, it was especially difficult to collect fresh fecal samples in these populations. Locations for each sample were recorded using global positioning system (Garmin Etrex Vista C, Garmin Ltd.).

Laboratory methods

Genomic DNA from skin samples was isolated using standard proteinase K digestion and phenol/chloroform extraction procedure (Sambrook et al. 1989), followed by a UNIQ-10 column (Sangon) purification. Fecal samples were extracted using QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer instruction. Extraction blanks were used as negative controls. We used 13 species-transferred microsatellite markers in this study (Table 1) (Slate et al. 1998, 2002) on the basis of consistently amplifying clear and polymorphic products in fecal samples. One homozygote of each locus was sequenced to confirm it was a short tandem repeat (STR) in Przewalski's gazelle. Then, forward primers of the 13 markers were fluorescently labeled with FAM, HEX and TAMRA (Table 1). In PCR process, we used a reaction volume of 10 ul, containing approximately 10 ng of genomic DNA, 0.2 μM of each primer, 0.1 mg/ml of bovine serum albumin (BSA, Biolabs) and 0.25 U HotStartTaq (QIAGEN). All PCR amplifications were carried out on a Thermo Hybaid MBS 0.2S cycler with an initial denaturation at 95°C for 15 min, followed by 40 cycles at 94°C for 45 s, 50°C for 30 s and 72°C for 45 s. Ending with a final extension at 72°C for 10 min, then held at 4°C. Negative controls were included with every PCR reactions to check for contamination. PCR products were resolved on an ABI PRISM 377 DNA Sequencer (Applied Biosystems). Alleles were scored using GENESCAN version 3.7 (Applied Biosystems) and GeneMarker version 1.71 (SoftGenetics).

Reliability of genotyping results

We conducted two replicate PCRs for skin samples. Fecal samples were amplified using a modified multiple-tube procedure (Taberlet et al. 1996). In practice, amplification

Table 1 Characteristics of 13 polymorphic microsatellite loci used in this study

Locus	Dye	Size range (bp)	No. of alleles	Primer sequence (5′–3′)
AF5	FAM	139–155	7	F:GTGGGAAGAGATAGAGGAAGC
				R:GAGCCACAAGGCACAGCCAAC
AGLA226	TAMRA	149–169	8	F:CTAAAGAAATGCAGTGTTGTCAGCC
				R:CTTAACAAGCCATGCTGAATGGTCT
BM1225	HEX	234–276	9	F:TTTCTCAACAGAGGTGTCCAC
				R:ACCCCTATCACCATGCTCTG
CSSM43	HEX	247–263	6	F:AAAACTCTGGGAACTTGAAAACTA
				R:GTTACAAATTTAAGAGACAGAGTT
IDVGA39	FAM	179–183	3	F:ACGGTGGGAACATCTTGTCACTA
				R:CCAGTATTCTTCCTGCGAAAAATC
JAB8	FAM	211–213	2	F:CACGTCACCCGCTTTCTCTTG
				R:GGTGAGTGTAACACCTGTGTGCG
RBP3	HEX	170–180	5	F:CTATGATCACCTTCTATGCTTCC
				R:CCCTAAATACTACCATCTAGAAG
RT1	FAM	210–228	7	F:TGCCTTCTTTCATCCAACAA
				R:CATCTTCCCATCCTCTTTAC
T156	TAMRA	136–156	5	F:TCTTCCTGACCTGTGTCTTG
				R:GATGAATACCCAGTCTTGTCTG
TEXAN15	HEX	204–222	6	F:TCGCAAACAGTCAGAGACCACTC
				R:TGGATGAGAAAGAAGAGCAGAGTTG
TGLA10	HEX	157–161	3	F:CTAAATTTATCCCACTGTGGCTCTT
				R:CAATCTGCAGTAGCATACATCCTTG
TGLA122	TAMRA	136–146	6	F:CCCTCCTCCAGGTAAATCAGC
				R:AATCACATGGCAAATAAGTACATAC
TGLA378	FAM	100–116	9	F:TTTATAGCCAACCATATACTTTGCC
				R:CAGTACCTCTCAACTTCATGTATGT

F forward primer, *R* reverse primer

was repeated minimal three times, when a heterozygote was observed in two separate reactions or a homozygote showed identical profile in three separate replicates, the alleles were recorded. Otherwise, we treated the alleles as missing data. Only samples with more than ten loci of allele data were included in statistical analyses. We calculated the probability of unrelated individuals or full-sibs bearing an identical multi-locus genotype [P_{ID} or $P_{ID(Sibs)}$] using GIMLET version 1.3.3 (Valière 2002). The fecal samples with identical alleles or with a single mismatch were considered to be from the same individual, then the repetitious samples were removed. The program MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) was used to check for microsatellite null alleles and scoring errors due to large allele drop-out or stuttering. Rates of genotyping error were calculated following the equations of Broquet and Petit (2004).

Genetic diversity analyses

The software GENEPOP version 4.0 (Raymond and Rousset 1995) was used to test for deviations from Hardy–Weinberg equilibrium (HWE) in each population and linkage disequilibrium (LD) between pairwise loci. Bonferroni corrections were applied to the HWE and LD tests involving multiple comparisons (Rice 1989). Since the samples were collected from 2004 to 2007, we performed an AMOVA analysis to check for the potential difference among samples collected in different years. The AMOVA analysis was conducted in Arlequin version 3.11 (Excoffier et al. 2005) through 10,000 permutations. We used GENETIX version 4.03 (Belkhir et al. 2004) to assess mean number of alleles per locus (MNA), expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (F_{IS}). The program FSTAT version 2.9.3.2 (Goudet 2002) was applied to calculate allelic richness (AR) by rarefaction analysis to account for uneven sample sizes (Petit et al. 1998). Private alleles (PA) in each population were counted by hand.

Population genetic structure analyses

The genetic structure of Przewalski's gazelle was investigated using two different Bayesian clustering methods, Structure and GENELAND. This could allow assessing the consistency and reliability of the results. First, we used Structure 2.0 software (Pritchard et al. 2000) to detect population genetic structure based on individual multilocus genotypes. Ten independent runs of K (number of hypothetic genetic groups) = 1–9 were performed at 1,000,000 Markov Chain Monte Carlo (MCMC) repetitions with a 100,000 burn-in period, using correlated allele frequencies and admixture model. K was identified using the maximum values of $Ln\ P(D)$ (the posterior probability of the data for a given K) and ΔK (the rate of change in the log probability of data between successive values of K) (Evanno et al. 2005). Second, we used GENELAND 3.1.4 program (Guillot et al. 2005) in R 2.8.1 (The R project for statistical computing), which incorporates geographical coordinate information for genotyped individuals, to estimate the most likely number of genetic groups (K) and their spatial boundaries. K was inferred from the modal value with the highest likelihood, and the spatial boundaries were detected as geographical areas which had low posterior probability of membership. We first ran ten independent MCMC chains for $K = 1$–9, using the spatial Dirichlet-model and the following parameters: 1,000,000 iterations with the first 10% as burn-in, sampling every 100, maximum rate of the Poisson process fixed to 169, maximum number of nuclei in the Poisson–Voronoi tessellation set to 507, uncertainty attached to spatial coordinates fixed to 2 km [approximately corresponding to the home range size of Przewalski's gazelle, Li (2008)]. Then, another ten independent runs were performed with the most likely K identified in the first step, using the same parameters. Maps of posterior probabilities of membership were compared among the independent runs to check the consistency of the results. In both Structure and GENELAND analyses, each individual was assigned to its most likely genetic group of ancestry; and then, for each sampled population, the proportion of individuals assigned to each genetic group was assessed.

We used F-statistics (Wright 1978) to estimate the level of genetic differentiation among the inferred genetic groups. The significance of pairwise F_{ST} values were assessed via 10,000 permutations using FSTAT. Migration rates among the inferred genetic groups were estimated using a non-equilibrium Bayesian method implemented in the program BayesAss version 1.3 (Wilson and Rannala 2003). We ran the program with 3×10^6 iterations, discarding the first 10^6 iterations as burn-in, and sampling the chain every 2,000 iterations. To test the consistency of the results, five independent runs were executed with different delta values and random seed (Wilson and Rannala 2003).

Demographic history analyses

Based on the assumption that a bottleneck population will show a significant excess of heterozygotes (Cornuet and Luikart 1996; Luikart et al. 1998), we used Bottleneck version 1.2.02 (Piry et al. 1999) to detect recent genetic bottlenecks in Przewalski's gazelle. Since the two-phase mutation model (TPM) is the most appropriate model for microsatellites (Di Rienzo et al. 1994; Piry et al. 1999),

we ran the program under TPM with 90% single-step mutations. The statistical significance of the results were determined using Wilcoxon sign-rank tests. We also used MSVAR 0.4.1 (Beaumont 1999; Storz and Beaumont 2002) to estimate demographic change. The program performs coalescent simulations using a Bayesian MCMC algorithm and estimates the posterior probabilities for demographic parameters, such as population growth rate ($r = N_0/N_1$, where N_0 = current effective population size, and N_1 = ancestral effective population size at the time of population decline or expansion), time since demographic change occurred ($t_f = t_a/N_0$, where t_a is the number of generations since the demographic change), and θ_{MSVAR} (= $2N_0\mu$, where μ is the mutation rate of the marker, and θ_{MSVAR} is used to calculate N_0). Each simulation was conducted with 20,000 thinned updates and a thinning interval of 10,000 steps, and using a user-predefined rectangular prior involving the three parameters [$log10(r)$, $log10(t_f)$, $log10(\theta_{MSVAR}) = -5$ to 5, which are sufficiently broad so that they would not affect the results (Storz and Beaumont 2002)]. The first 10% updates were removed as burn-in, and the remaining data yielded lower (5%), median (50%) and upper (95%) quantities of the posterior distributions of the parameters. Five independent simulations were executed with both exponential and linear models. The mutation rate (μ) of microsatellite was assumed as 5×10^{-4} per generation (Goldstein and Schlötterer 1999; Schlötterer 2000; Whittaker et al. 2003), and the generation time of Przewalski's gazelle was considered three years (Jiang 2004). All demographic analyses were conducted on the genetic groups inferred in this study. Genetic group G4 (namely P7, Bird Island) was ignored in the demographic analyses because of small sample size.

Results

Reliability of genotyping results

Totally, 28 skin samples and 182 fresh fecal samples were collected in the field. In the laboratory, 25 skin samples and 161 fecal samples were amplified with more than ten loci. The unbiased P_{ID} of 3.48×10^{-9} and $P_{ID(Sibs)}$ of 2.63×10^{-4} indicate high reliability of the 13 microsatellite markers to distinguish fecal samples from the same individual. After eliminating repetitious samples, we obtained genotype data of 169 individuals (Table 2). The total genotyping error rates were 0.328%, indicating high reliability of the data. MICRO-CHECKER did not detect the presence of null alleles or scoring errors.

Genetic diversity

For each population and the entire sample, all microsatellite loci were in HWE ($P > 0.05$). There are 37 out of 702 loci pairs for each population (P1, 2; P2, 5; P3, 10; P5, 2; P6, 6; P8, 3; P9, 9) and seven out of 78 loci pairs for the entire sample showing significant LD. However, significant LD no longer existed after Bonferroni corrections. The result from AMOVA anaysis indicated that there was no difference among samples collected in different years (Table 3). A total of 76 alleles were detected from 13 microsatellite loci, ranging from two to nine alleles per locus (Table 1). Mean allele number across all populations was 5.85, and overall H_O and H_E were 0.525 and 0.552, respectively (Table 2). Allelic richness of the entire sample was 5.84, and 13 private alleles were found in three populations (Table 2). The values of F_{IS} did not obviously deviate from zero except P4 (−0.233) and P7 (0.120)

Table 2 Genetic diversity of Przewalski's gazelle using 13 microsatellite loci

Sampling population	N	MNA	H_E	H_O	AR	PA	F_{IS} (95% CI)
P1	24	4.00	0.574	0.579	2.23	0	−0.008 (−0.118 to 0.053)
P2	38	3.85	0.474	0.500	2.01	0	−0.056 (−0.139 to −0.009)
P3	32	3.85	0.462	0.459	1.97	0	0.006 (−0.103 to 0.074)
P4	3	2.31	0.490	0.577	2.00	0	−0.233 (−1.000 to −0.233)
P5	8	3.38	0.532	0.533	2.14	0	−0.001 (−0.229 to 0.049)
P6	19	3.62	0.532	0.547	2.13	0	−0.028 (−0.167 to 0.068)
P7	3	2.92	0.626	0.564	2.41	1	0.120 (−1.000 to 0.120)
P8	21	4.15	0.554	0.570	2.18	3	−0.030 (−0.146 to 0.032)
P9	21	3.85	0.564	0.534	2.22	9	0.053 (−0.078 to 0.123)
Total	169	5.85	0.552	0.525	5.84	13	−0.014 (−0.028 to 0.002)

N number of individuals, *MNA* mean number of alleles, H_E expected heterozygosity, H_O observed heterozygosity, *AR* allelic richness, *PA* private alleles, F_{IS} inbreeding coefficient, CI confidence interval

Table 3 Result of AMOVA analysis on the samples collected in different years

Source of variation	Fixation index	Percentage of variation	*P* value
Among samples collected in different years	$\Phi_{CT} = 0.023$	2.26	0.171
Among samples collected in the same year	$\Phi_{SC} = 0.009$	0.91	0.371
Within samples	$\Phi_{ST} = 0.032$	96.84	0.025

$P < 0.05$ indicates statistical significant

(Table 2), and all F_{IS} values had no statistical significance ($P > 0.197$).

Population genetic structure

In the structure analyses, average *Ln P(D)* maximized at $K = 6$ genetic groups consisting of: G1 (P1), G2 (P2, P3, P4), G3 (P5, P6), G4 (P7), G5 (P8) and G6 (P9) (Tables 4, 5), and the sampled populations had an estimated proportion of membership ranging from 0.66 to 0.87 (Table 5). The highest value of average ΔK emerged at $K = 3$ genetic groups containing: G′1 (P1, P5, P6, P7, P8), G′2 (P2, P3, P4) and G′3 (P9) (Tables 4, 5), with the estimated proportion of membership varying between 0.69 and 0.91 (Table 5). Because the main incongruence of the results was that there were four small genetic groups (G1, G3, G4, G5) or one large genetic group (G′1), we re-ran the data of G′1 to test for further subdivision. As a result, four genetic groups (the same as G1, G3, G4, G5) were detected within G′1 (Table 4). Altogether, the results from structure analyses indicated that there were most likely six genetic groups (G1–G6) within Przewalski's gazelle. GENELAND

Table 4 Results of structure analyses on the entire sample and on the data of G′1

The entire sample			The data of G′1		
K	*Ln P(D)*	*ΔK*	*K*	*Ln P(D)*	*ΔK*
1	−4787.8	–	1	−2196.1	–
2	−4659.1	1.2	2	−2177.7	0.4
3	−4517.1	**120.3**	3	−2162.5	2.6
4	−4476.9	12.6	4	**−2128.7**	**8.9**
5	−4458.1	4.8	5	−2142.7	–
6	**−4422.5**	62.8			
7	−4496.6	1.8			
8	−4583.3	1.5			
9	−4722.2	–			

Average *Ln P(D)* and *ΔK* values across ten runs are shown for each *K*

Values in bold type indicate maximum *Ln P(D)* and *ΔK*

Table 5 The estimated proportion of membership to the genetic groups inferred by structure and GENELAND for each sampled population

Sampling population	Structure [*Ln P(D)*]						Structure [*ΔK*]			GENELAND					
	G1	G2	G3	G4	G5	G6	G′1	G′2	G′3	G1	G2	G3	G4	G5	G6
P1	**0.66**	0.08	0.11	0.04	0.09	0.02	**0.73**	0.23	0.04	**0.86**	0.03	0.03	0.05	0.02	0.01
P2	0.11	**0.66**	0.10	0.02	0.08	0.03	0.13	**0.84**	0.03	0.02	**0.90**	0.03	0.02	0.02	0.01
P3	0.07	**0.67**	0.12	0.03	0.08	0.03	0.13	**0.84**	0.03	0.02	**0.88**	0.04	0.01	0.03	0.02
P4	0.06	**0.71**	0.07	0.01	0.11	0.04	0.12	**0.86**	0.02	0.03	**0.88**	0.03	0.02	0.01	0.03
P5	0.09	0.10	**0.72**	0.02	0.04	0.03	**0.70**	0.20	0.10	0.02	0.03	**0.90**	0.02	0.02	0.01
P6	0.12	0.09	**0.66**	0.03	0.08	0.02	**0.69**	0.27	0.04	0.01	0.02	**0.89**	0.03	0.02	0.03
P7	0.02	0.05	0.10	**0.69**	0.11	0.03	**0.69**	0.28	0.03	0.24	0.02	0.03	**0.66**	0.04	0.01
P8	0.05	0.04	0.03	0.02	**0.83**	0.03	**0.89**	0.08	0.03	0.02	0.02	0.02	0.02	**0.91**	0.01
P9	0.02	0.05	0.02	0.01	0.03	**0.87**	0.02	0.07	**0.91**	0.02	0.02	0.01	0.01	0.01	**0.93**

Values in bold type indicate the highest proportion of membership for each population

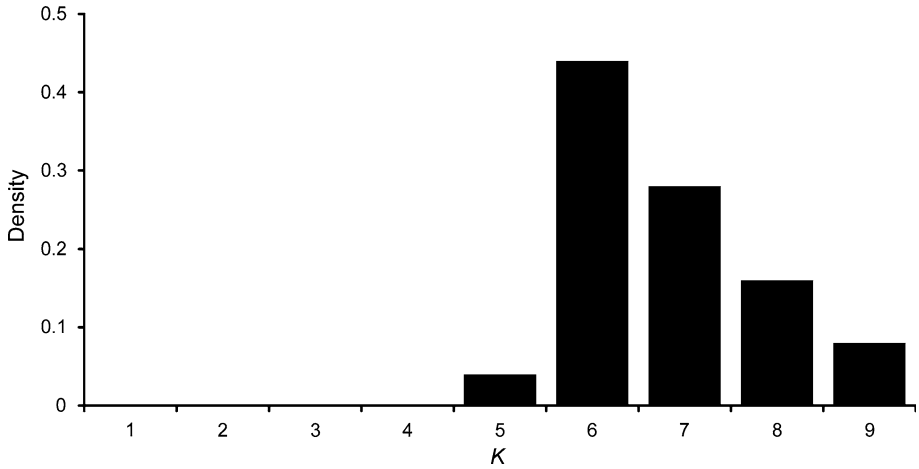

Fig. 2 Histogram from GENELAND analyses showing the average density of inferred *K* across ten runs

analyses gave consistent results among independent runs. The results suggested six spatial genetic groups across the whole data (Figs. 2, 3, 4), in accordance with the Structure results. The probability of membership for each sampled population ranged from 0.66 to 0.93 (Table 5). Genetic differentiation (F_{ST} value) among the inferred genetic groups was significant and ranged from 0.013 to 0.117 (Table 6). BayesAss analyses showed constant results, which revealed low migration rates among the inferred genetic groups (Table 7).

Population demographic history

We found evidence of recent genetic bottlenecks (denoted by heterozygosity excess, $P < 0.05$) in four genetic groups (G1, $P = 0.0002$; G2, $P = 0.0107$; G3, $P = 0.0034$; G6, $P = 0.0002$), indicating significant population declines of Przewalski's gazelle in recent years (usually over a few dozen generations, Luikart et al. 1998). In MSVAR analyses, five independent replicates showed concordant results. Sharp population declines were detected by the posterior distribution of N_0 and N_1, with the ancestral effective population sizes decreasing to less than one percent (Table 8). Under the exponential model, we detected relatively recent population decline (within 400 years) in each inferred genetic group (Table 8). Under the linear model, even longer population declines were found (Table 8).

Discussion

Genetic diversity

Our results revealed moderate nuclear genetic diversity in Przewalski's gazelle (Table 2), suggesting that this endangered gazelle still has evolutionary potential. However, when compared with genetic variation of other wild gazelles using the least biased estimator (H_E), such as Grant's gazelle (0.61, Arctander et al. 1996), Chiru (0.84, Zhou et al. 2007) and goitered gazelle (0.72, Zachos et al. 2010), the nuclear genetic diversity of Przewalski's gazelle is relatively low. Previous genetic study on Przewalski's gazelle using mtDNA also found that the nucleotide diversity (<0.004) of Przewalski's gazelle is lower than most mammals (Lei et al. 2003a). Recent genetic bottleneck and severe population decline may account for the erosion and depletion of genetic diversity in Przewalski's gazelle. The results from private allele analyses indicated that population P7, P8 and P9 had some extent of genetic distinctiveness (Table 2), thus they should receive more conservation attentions.

Population genetic structure

The structure and GENELAND analyses found six genetic groups in Przewalski's gazelle (Figs. 2, 3, 4; Tables 4, 5). Between the inferred genetic groups, significant genetic differentiation and low migration rates were detected (Tables 6, 7). Geographical distance might partly account for the observed population genetic structure (especially for distant populations), because there was no record of long-distance dispersal or migration of the gazelle (Li et al. 1999) and three distant populations (P7, P8 and P9) each formed a genetic group (G4, G5 and G6). Moreover, since human population in the study area increased ten times in the past hundred years (National Census Data), fragmenting the habitat of the gazelle, anthropogenic factors might be also partly responsible for the observed genetic structure (especially for close populations). For example, P1 and P2 are only 12 km apart, yet they belong to different genetic groups. Given that the gazelle has high locomotive ability (45–55 kph) and a 20 km migration of the gazelle in a barrier-free area was recorded (Li et al. 1999), the human settlements and roads between P1 and P2 could be the main

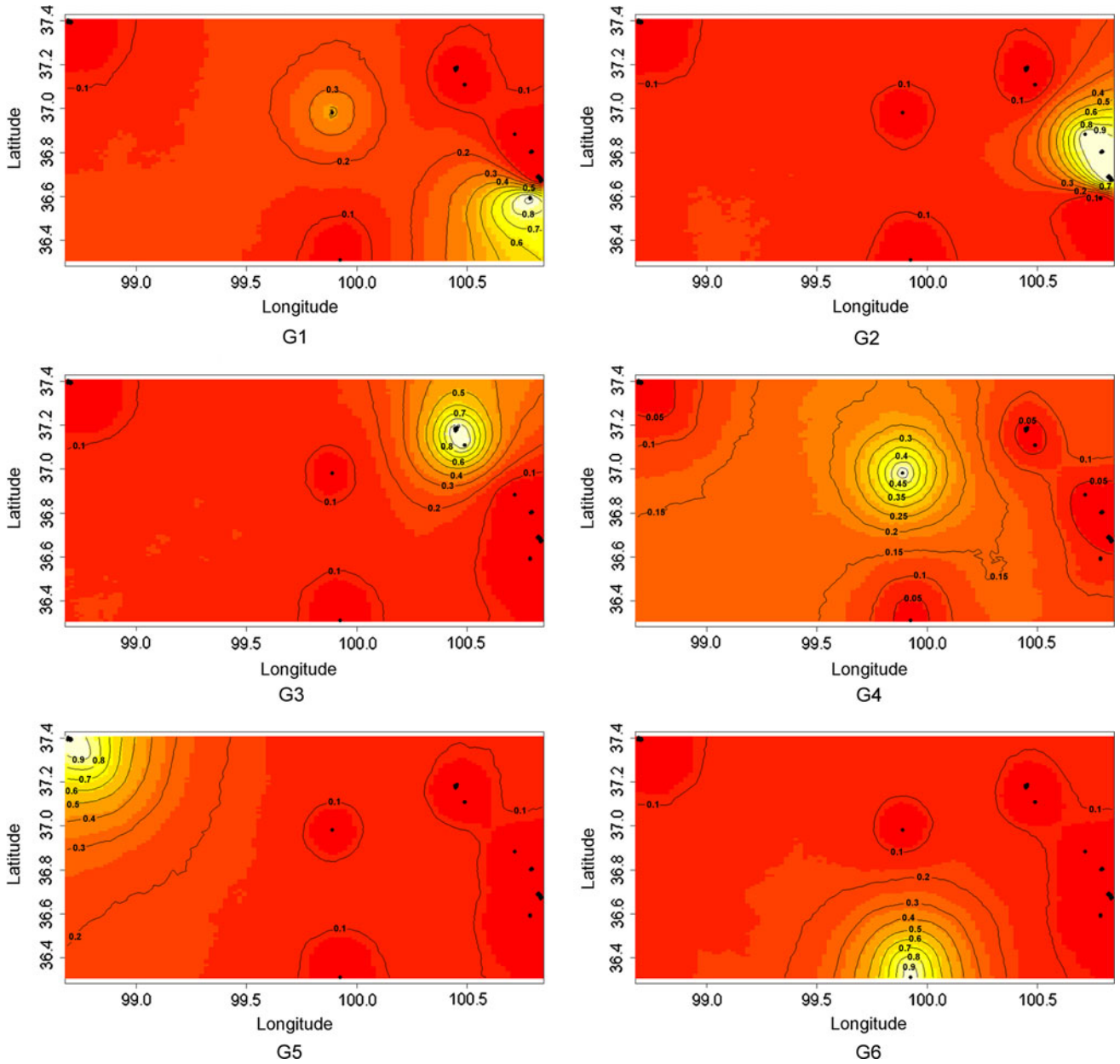

Fig. 3 Maps of posterior probabilities belonging to one of *K*= six spatial genetic groups (G1–G6) for 169 individuals of Przewalski's gazelle. *Black sites* represent sampling populations. *White areas* indicate the highest probability of membership of the inferred genetic groups

influencing factors. In contrast, other close populations with no potential barriers to movements among them, such as P2 and P3, were assigned to the same genetic group. Additionally, the genetic differentiation between P9 and other populations was probably affected by Qinghainanshan Mountain (Fig. 1). Former research on Przewalski's gazelle using mtDNA control region sequences (Lei et al. 2003a) revealed a population genetic structure (Yuanzhe—P1 in this study, Hudong-Ketu—P2 and P3 here, Shadao-Gahai—P4 here, and Bird Island—P7 here) largely consistent with this study. One difference is that P4 was inferred as an independent genetic group by Lei et al. (2003a).

Population demographic history

Several publications have stated that Przewalski's gazelle has experienced a rapid population decrease (Jiang et al. 1995, 2000). However, no attempt has been made to estimate the extent and initial time of the population decline. In our study, the results from MSVAR analyses provide strong evidences for significant population declines under

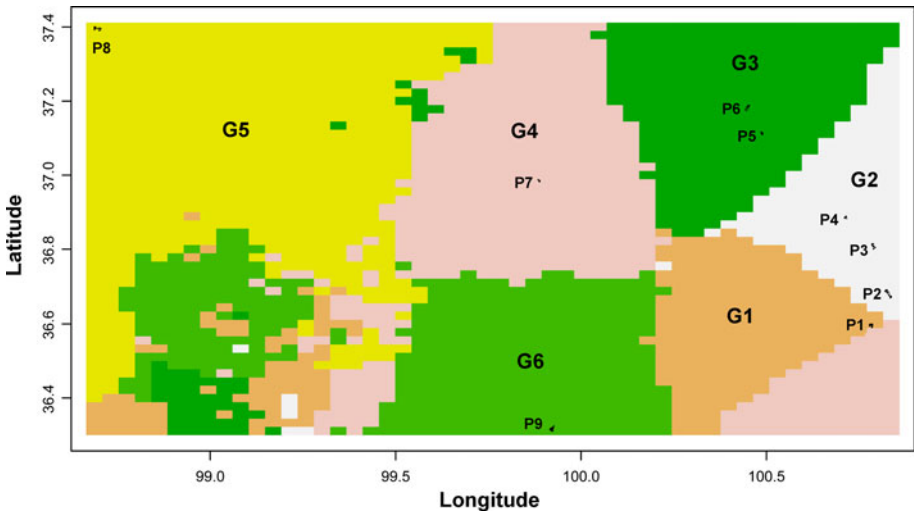

Fig. 4 Synthetic map of population membership showing the ranges of the six inferred genetic groups. *Black sites* indicate sampling populations

Table 6 Genetic differentiation (F_{ST}) between the six inferred genetic groups

Genetic group	G1	G2	G3	G4	G5	G6
G1	–					
G2	0.064**	–				
G3	0.051**	0.067**	–			
G4	0.013*	0.097**	0.060*	–		
G5	0.071**	0.088**	0.097**	0.040*	–	
G6	0.086**	0.117**	0.085**	0.064**	0.104**	–

* $P < 0.05$, ** $P < 0.01$

Table 7 Migration rates between the six inferred genetic groups from BayesAss analyses

Genetic group	G1	G2	G3	G4	G5	G6
G1	–	0.054 (±0.023)	0.047 (±0.010)	0.057 (±0.016)	0.055 (±0.025)	0.037 (±0.014)
G2	0.031 (±0.015)	–	0.036 (±0.014)	0.038 (±0.011)	0.033 (±0.015)	0.029 (±0.007)
G3	0.030 (±0.012)	0.034 (±0.017)	–	0.033 (±0.009)	0.033 (±0.010)	0.027 (±0.009)
G4	0.028 (±0.011)	0.034 (±0.016)	0.032 (±0.016)	–	0.034 (±0.013)	0.029 (±0.012)
G5	0.033 (±0.018)	0.040 (±0.013)	0.034 (±0.008)	0.041 (±0.015)	–	0.030 (±0.011)
G6	0.056 (±0.017)	0.056 (±0.024)	0.046 (±0.012)	0.056 (±0.022)	0.052 (±0.019)	–

Values in the parentheses represent standard deviation. The direction of migration is from the population below diagonal to the population above diagonal

both exponential and linear models, with the ancestral effective population sizes reducing to less than one percent (Table 8). The exponential model inferred that the population declines occurred within the last 400 years (Table 8), largely consistent with the results of Bottleneck analyses which indicated significant population reduction within a few dozen generations. Although the linear model suggested very small N_0 and that the population reduction started more than 2000 years ago (Table 8), the results should be treated with caution because the linear model is considered to be more applicable to "open" populations (Storz and Beaumont 2002), whereas Przewalski's gazelle is restricted to isolated populations (Jiang et al. 1995, 2000; Table 7). It is difficult to exactly pinpoint why the population decline started because there is no evidence to eliminate anthropogenic, ecological, climatic or other explanations. However, based on local chronicles of the four counties in the study area (Gonghe County Chronicles Compilation Committee 1991; Haiyan County Chronicles Compilation Committee 1994; Tianjun County Chronicles Compilation Committee 1995; Gangcha County Chronicles Compilation Committee 1998) and other literatures (Hu 2005; Mi 2007; Wang 2009), we infer that the population decline of Przewalski's gazelle was probably tied to the development of human civilization in the study area. For one thing, the population decline of the gazelle

Table 8 Posterior distributions of N_0, N_1 and T (when population decline began) inferred from MSVAR under the exponential and linear model

Genetic group	N_0 50% (5% to 95%)	N_1 50% (5% to 95%)	T (years) 50% ± SD (GT = 3)
Exponential model			
G1	12 (1–314)	6005 (3,151–11,615)	48 ± 12
G2	69 (8–409)	8837 (2,905–15,433)	340 ± 56
G3	16 (1–261)	7429 (3,473–14,269)	74 ± 21
G5	55 (1–898)	9809 (5,457–25,137)	275 ± 38
G6	52 (2–646)	5793 (2,600–15,866)	236 ± 33
Linear model			
G1	4 (1–121)	5996 (3,597–12,318)	670 ± 73
G2	4 (1–63)	5610 (3,769–10,071)	669 ± 81
G3	5 (1–99)	8050 (4,720–12,489)	934 ± 105
G5	8 (1–320)	11606 (7,076–23,070)	1672 ± 154
G6	28 (1–442)	8397 (3,918–14,683)	2178 ± 221

The posterior distributions of N_0 and N_1 were described by the 5, 50, and 95% quantile computed across five replicates. The posterior distributions of T were described by the 50% quantile with standard deviation (± SD) across five replicates. T was estimated using a generation time (GT) of three years

may be associated with the expansion of human colonization. Human colonization began to increase in this area during the Han Dynasty (1,800 to 2,200 years ago), then colonization particularly accelerated since the Qing Dynasty (360 years ago), with a more than tenfold increase of human population size. We can see that, approximately at the time when human colonization accelerated, the gazelle population began to significantly decrease. For another, the study area was a war zone as war relics can be easily found across the landscape (Wang 2009). The wars frequently broke out since the Han Dynasty, with the war period of approximate twenty years (Qinghai Province Chronicles Compilation Committee 2001). It was possible that soldiers hunted gazelles for food during the war time, so historical military conflicts probably influenced the population demography of the gazelle.

Conservation implications

Based on the results of this study, we propose the following measures to conserve Przewalski's gazelle. First, since the gazelle currently possesses moderate genetic diversity and experienced genetic bottleneck and severe population decline, we recommend that periodic monitoring for all populations should be conducted in order to hold the population trends in time. Moreover, effective conservation measures such as habitat restoration should be carried out to facilitate the increase of population size, and ultimately, to counteract the negative effect of genetic bottleneck. Second, the six genetic groups identified in this study should be considered as MUs following the criterions of Moritz (1994). Because of the genetic distinctiveness and far distance from other populations, population P7, P8 and P9 each should be managed as separate MU. Although the inter-population distances of other six populations (P1−P6) are short, they should be also managed separately according to the three inferred genetic groups (G1−G3) so as to maintain genetic diversity. Third, special conservation consideration should be given to P9. Population P9 has most genetic distinctiveness (nine private alleles) among all populations, it is the only population locating in the Gonghe Basin and is isolated from other populations by Qinghainanshan Mountain. In addition, other options such as periodic individual translocation and ex situ conservation are available, but we need more biological data of this species before these options become viable alternatives.

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