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FULL PAPER

A preliminary study of the genetic diversity of Xinjiang Tarim red deer (*Cervus elaphus yarkandensis*) using the microsatellite DNA method

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Abstract

To evaluate the genetic diversity of the Xinjiang Tarim red deer (*Cervus elaphus yarkandensis*) population, we analyzed the frequencies of microsatellite alleles. Samples were collected from 3 isolated populations in Xaya, Lopnur and Qarqan of Xinjiang. Although 10 microsatellite loci were examined, alleles of 133 to 190 base-pairs were detected for only 3 loci : BM5004, BM4208 and BM888. The average observed multilocus heterozygosity was 0.08 ± 0.04 for the Xaya, 0 for the Lopnur, and 0.17 ± 0.08 for the Qarqan population. The average heterozygosity of all populations was 0.08 ± 0.02. The observed heterozygosities were significantly lower than the expected values. The present results suggest that the bottleneck effect has occurred in the populations of the Xinjiang Tarim red deer.

Key words : *Cervus elaphus yarkandensis*, microsatellite DNA, Tarim deer, Xinjiang

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Introduction

The Tarim red deer (*Cervus elaphus yarkandensis*) inhabits the river valleys of the Tarim, Konqi and Qarqan rivers in Xinjiang, China (Fig. 1). Due to its limited distribution and population size, the Tarim deer has been included in the Red List (1994) of the International Union for the Conservation of Nature and Natural Resources (IUCN) in the category of an endangered species and classified as a second class protected animal in China. Currently, the habitat of the Tarim deer is an area of intensive human activity and the deer population become isolated into three areas, namely Xaya, Lopnur and Qarqan. Luo and Gu[10] reported that the Tarim deer population is on the verge of extinction and that the number of individuals is decreasing in each area. So far, the protective measures that have been implemented regarding the Tarim deer have not been sufficiently effective, and the ecology and genetic features of this species remain to be elucidated.

Detailed knowledge of the reproduction and the genealogical relationships among individuals is essential to developing effective measures to protect endangered animals. An especially serious problem is caused by population bottlenecks: a severe reduction of population size, which reduces the diversity of a population and its genetic variability. This makes animals especially vulnerable to changes in their environment and to diseases.

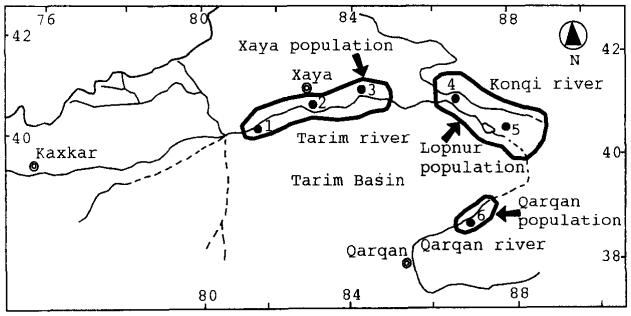

Fig. 1. Locations (1. Alar, 2. Xaya, 3. Bugur, 4. Lopnur, 5. Tikanlik, 6. Aktaz) of the sampled populations of the Tarim red deer (*Cervus elaphus yarkandensis*) in Tarim basin, Xinjiang, China.

Microsatellite analysis makes it possible to determine the genotypes of individuals by examining the variation of the number of repetitions of sequences that are several nucleotides long. The mutation rate of a given microsatellite locus is considered to be 10^{-4}~10^{-5}per generation[4]. Microsatellite DNA is used as a marker of DNA polymorphism that is an effective hereditary index in ecological, evolutionary and genetic studies[3,5,7]. Microsatellite analysis has been used for the study of genetic structures and reproductive success in deer species[11,13,14,20]. In the present study, we obtained preliminary information about the genetic diversity in the Tarim deer populations using microsatellite polymorphisms.

Materials and Methods

Sample collection

The samples for the DNA analysis were collected during a field survey in September 2000 in the Tarim basin, Xinjiang. The deer hair samples were collected from both wild and captive Tarim deer from 3 isolated populations (Fig. 1), namely, those in Xaya (9 animals), Lopnur (5 animals) and Qarqan (4 animals).

DNA extraction

DNA was extracted from the hairs using the chelex protocol[21].

PCR

In the present study, 10 sets of primers were used as diagnostic markers (Table1). All of these sets were designed based on sequence information from the genomes of bovine and ovine species and applied to genetic studies of

Table 1. PCR primer sequences for microsatellite DNA analysis used in the present study

Microsatellite locus	Primer sequence (5′-3′)	Reference
BM203	GGGTGTGACATTTTGTTCCC	Bishop et al. (1994)[1]
	CTGCTCGCCACTAGTCCTTC	
BM848	TGGTTGGAAGGAAAACTTGG	Bishop et al. (1994)[1]
	CCTCTGCTCCTCAAGACAC	
BM888	AGGCCATATAGGAGGCAAGCTT	Bishop et al. (1994)[1]
	CTCGGTCAGCTCAAAACGAG	
BM4107	AGCCCCTGCTATTGTGTGAG	Bishop et al. (1994)[1]
	ATAGGCTTTGCATTGTTCAGG	
BM4208	TCAGTACACTGGCCACCATG	Bishop et al. (1994)[1]
	CACTGCATGCTTTTCCAAAC	
BM5004	TCTGGAGTGAATGTTTCTGAGG	Bishop et al. (1994)[1]
	TTGTGATGAGCACCTGAAGG	
BMC1009	GCACCAGCAGAGAGGACATT	Bishop et al. (1994)[1]
	ACCGGCTATTGTCCATCTTG	
ETH152	TACTCGTAGGGCAGGCTGCCTG	Steffen et al. (1993)[18]
	GAGACCTCAGGGTTGGTGATCAG	
OarFCB193	TTCATCTCAGACTGGGATTCAGAAAGGC	Buchanan and Crawford (1993)[2]
	GCTTGGAAATAACCCTCCTGCATCCC	
VH110	CTCTAGAGGATCACAGAGAGTCGG	Hanrahan et al. (1993)[8]
	GCAGAAACATTTTTTTCCTTCAATATAGTTTCCC	

Cervidae[9,14,16,17,19]. The sets used were : BM203, BM848, BM888, BM4107, BM4208, BM5004, BMC1009, ETH152, OarFCB193, and VH110. The polymerase chain reaction (PCR) amplification was performed using Gene Amp PCR System 9700 (PE Byosystems). The PCR was performed in a total volume of 50 μl, using the primer pair and 20 ng of genomic DNA. The reaction mixture contained 0.8 μM [F]-dCTP and 5 units of TaKaRa Taq DNA polymerase (Takara, Japan) in buffer consisting of 10mM Tris-HCl(pH8.3), 50mM KCl, and 1.5 mM $MgCl_2$. TAMRA-dCTP (yellow, 100μM) was obtained from ABI/PEC (product number 402793). PCR was performed using 35 cycles of a series of incubations at 94 ℃(denaturation) for 1 min, 45-50℃(annealing) for 1 min, and 72℃(extension) for 1min. The annealing temperature was reduced to 45℃ for OarFCB 193, 46℃ for BM848 and VH110, 47 ℃ for BM 203, BM888, BM5004 and ETH152, and 50℃ for BM4107, BM4208 and BMC1009 marker amplification. A final extension step was performed at 72℃ for 10 min.

Genotyping

An aliquot (0.5 μl) of the PCR products was mixed with 2.5 μl of deionized formamide, 0.5 μl of Blue Dextran/ethylenediaminetetraacetic acid(EDTA), and 0.5 μl of GeneScan 1000 Red Dye(ROX) marker. The mixture was then loaded on a 6% denaturing polyacrylamide gel(ABI PRISM Geluxe 377-36 WTR) and electrophoresed for 2 hr using a DNA sequencer ABI-PRISM 377.

Data analysis

Genetic polymorphism was expressed as the number of alleles per locus, while the observed heterozygosity (Ho) and expected heterozygosity (He ; based on the Hardy-Weinberg assumption) were calculated using the

GENEPOP package, version 3.1b[15]. This program was also used to test for deviations from Hardy-Weinberg equilibrium within a population at a given locus and over all loci.

Results

Among the 10 microsatellite loci examined, clear bands of 133 to 190 base-pairs representing alleles were detected for only 3 loci, namely BM5004, BM4208 and BM888. At the BM5004 locus, 2 alleles were found in all 3 populations. At the BM4208 locus, 4 alleles were found in Xaya, 3 in Lopnur and 1 in Qarqan. At the BM888 locus, 2 alleles were found in Xaya and single bands were observed in Lopnur and in Qarqan. At least 1 common allele was found for each locus in all 3 populations of Tarim deer. Table 2 shows the allele frequencies and observed heterozygocities of the 3 loci. The average observed multilocus heterozygocity was 0.08±0.04 for the Xaya, 0 for the Lopnur, 0.17±0.08 for the Qarqan population.

The average heterozygosity of all populations was 0.08 ± 0.02.

For the other 7 loci examined (BM203, BM848, BM4107, BMC1009, ETH152, OarFCB 193 and VH110), no allele were identified, probably due to mismatching of primer sequences or due to null alleles.

Table 2. Microsatellite variation in the Tarim red deer (*Cervus elaphus yarkandensis*) populations

	Locus		Xaya	Lopnur	Qarqan	Total
BM5004	No. of individuals		9	5	4	18
	Allele[a] & frequency	133	0.833	0.400	0.375	0.611
		137	0.167	0.600	0.625	0.389
	ho[b]		0.111	0	0.250	0.111
	he[c]		0.278	0.480	0.469	0.475
BM4208	No. of individuals		7	5	4	16
	Allele[a] & frequency	144	0	0.200	0.250	0.125
		148	0.571	0.200	0	0.313
		150	0.357	0.600	0.750	0.531
		162	0.071	0	0	0.031
	ho [b]		0.143	0	0	0.063
	he [c]		0.541	0.320	0.375	0.604
BM888	No. of individuals		9	5	4	18
	Allele[a] & frequency	200	1	1	0.875	0.972
		198	0	0	0.125	0.028
	ho[b]		0	0	0.250	0.056
	he[c]		0	0	0.219	0.054
Average over	No. of individuals		8.33±0.67	5.00±0.00	4.00±0.00	17.3±0.7
3 loci±SE	A		0.38±0.14	0.38±0.12	0.38±0.12	0.38±0.12
	Ho[b]		0.08±0.04	0	0.17±0.08	0.08±0.02
	He[c]		0.27±0.16	0.27±0.14	0.35±0.07	0.38±0.17

[a] Molecular sizes (bases) refer to allele name.

[b] ho and Ho : observed heterozygosity.

[c] he and He : expected heterozygosity.

Discussion

The Tarim red deer lives in a desert landscape and its morphology differs from that of other red deer. Due to the poor condition of its habitat and the influence of recent agricultural expansion, very few Tarim deer have survived. Gao and Gu[6] reported that only 15,000 of these deer were surviving in the Tarim basin. Recent studies showed that the population of Tarim deer in Qarqan does not number more than 50[10], while there are around 200 deer in Lopnur and Xaya. The habitat fragmentation in the Tarim basin is thought to influence the effective population size of this deer. However, little is known about the genetic status of the local populations. Studies of the genetic variations within a population of the Japanese sika deer[12,20] or the North American wapiti[14] revealed that microsatellite DNA analysis is very useful for detecting population bottlenecks, inbreeding and other genetic parameters of deer populations. In the present study, we tried to find polymorphic loci that would be useful for monitoring genetic diversity of deer populations. Only 3 of 10 primer sets used provided clear results. The other 4 sets were hot informative although they had been informative in other deer species[11,13,14,20]. The low level of amplification obtained in the present research may have been due to mismatching of primer sequences or to null alleles.

The limited number of samples used in the present study was not sufficient to warrant broader conclusions about the level of inbreeding or genetic bottleneck. The results showed that the observed heterozygosity was lower than that expected, with the exception of BM888 at Qarqan. This reduced heterozygosity suggests that the population of Tarim deer in Xinjiang has passed through bottleneck(s), and, as mentioned above, that the population size of Tarim deer has been reduced.

The mean heterozygosity of the Tarim red deer population was Ho=0.08±0.02. By comparison, the wapiti have a higher observed mean heterozygosity, Ho=0.552±0.039, at the BM5004, BM4208 and BM888 loci[19]. The low Ho of Tarim red deer may result from inbreeding due to population subdivision and shrinkage. In order to more precisely evaluate the genetic diversity of the Tarim red deer, we will have to obtain more microsatellite data.

Genetic research provides information that is important for establishing conservation plans for endangered species. It is essential to accumulate further genetic data about Tarim red deer in order to make an effective conservation plan.

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