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Analysis on Genetic Diversity of Reindeer (*Rangifer tarandus*) in the Greater Khingan Mountains Using Microsatellite Markers

Jian-Cheng Zhai[1], Wei-Shi Liu[1], Ya-Jie Yin[2], Yan-Ling Xia[1], and He-Ping Li[1,*]

[1]*College of Wildlife Resources, Northeast Forestry University, Harbin 150040, China. E-mail: zhaijc@nefu.edu.cn (Zhai); liuweishi@nefu.edu.cn (Liu); xiayanling@nefu.edu.cn (Xia)*

[2]*College of Bioengineering, Daqing Normal University, Daqing 163712, China. Email: niecy1978@dqsy.net*

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Jian-Cheng Zhai, Wei-Shi Liu, Ya-Jie Yin, Yan-Ling Xia, and He-Ping Li (2017) The only population of reindeer (*Rangifer tarandus*) in China, herded extensively by the Ewenki people, is the most southern population in the world. Genetic diversity plays a key role in the survival of endangered reindeer. To systematically understand the genetic variability of reindeer in China, 163 individuals from 8 populations were analyzed using 11 microsatellite loci. A total of 85 alleles were detected and the average number of alleles per locus was 7.7. The observed heterozygosity and expected heterozygosity ranged from 0.3736 to 0.5299 and from 0.6491 to 0.7608. Hardy-Weinberg equilibrium analysis indicated that a deficiency of heterozygotes existed in all eight populations. Both the F_{ST} and AMOVA analyses showed a low level of genetic differentiation among populations. UPGMA dendrogram revealed that population SYL formed one cluster, separating from the other populations. Then the GWQ and YSH populations formed another cluster and clustered with the BDX, BLJY, DML, DW and MLYS populations. Increasing the current exchange rate of reindeer among different populations and establishing natural reserve may be the effective approaches to conserve the fragile reindeer populations in China.

Key words: Reindeer (*Rangifer tarandus*), Genetic variability, Microsatellite markers, Genetic diversity, Genetic differentiation.

BACKGROUND

Reindeer (*Rangifer tarandus*) is a widespread and circumpolar animal in the world. They are mainly distributed throughout the northern part of the Arctic region, including arctic and sub-arctic region of Asia, Europe and the North American (Røed et al. 2010; Williams and Heard 2010). Reindeer is listed as a species of “least concern” on the Red List of Threatened Species of the World Conservation Union (IUCN) (Henttonen and Tikhonov 2008).

However, reindeer is also found in Genhe, north of Greater Khingan Mountains in Inner Mongolia, in China (Li and Bai 2004; Yin et al. 1999). In China, reindeer is an alien species that migrated gradually into the north of Greater Khingan Mountains in Inner Mongolia from the Lena River Basin of West Siberia region and the Lake Baikal Basin of Yakutia Republic by the Ewenki peoples who coexists with the reindeer, for the purpose of escaping during fighting in 17th century. Consequently, reindeer in China belong to the Siberia woodland reindeer subspecies (*Rangifer tarandus valintinae*) (Zhong et al. 2008). At present, Genhe is the only area to support a reindeer population in China, where reindeer is semi-domesticated by endemic local Ewenki people, a minority nationality who depends on the reindeer for food and livelihood for more than 300 years. As the most southern population in the world, reindeer in China have lost the habit of migration along with

*Correspondence: Jian-Cheng Zhai and Wei-Shi Liu contributed equally to this work. Tel: 0451-82190389.
E-mail: lihepinghrb2002@nefu.edu.cn

the semi-domestication. Furthermore, reindeer play an important role in economic and cultural development of the Ewenki people in China.

Since 1949, the founding for the People's Republic of China, the Communist Party and the People's Government have paid close attention to the protection and development of reindeer resources, but the situation of reindeer is not stable and optimistic. The range of reindeer is only across the region E121°00'-122°31', N50°40'-51°51'. Reindeer are managed by 8 hunter points in different locations, factors such as mountains and traffic have restricted the movement of reindeer between locations, and the mixing phenomenon barely occurs, limiting gene exchange. The population of reindeer averaged merely around 800 (standard error ± 120) from 1970 to 2014, and reaching a maximum of 1080 in 1982, a minimum of 463 in 1998 (Meng et al. 2014). At present the range of activities is less than 70 hm^2, the population size is only more than 600, and it has been endangerment or even extinction, facing a great test. Isolated small populations and fragmented habitat induced by natural barrier and regional activities (roads and towns), together with extensive management, are exposed to loss of genetic variability which can lead to reduced fitness, inbreeding and in some cases, extinction of the breed in some cases (Apps and McLellan 2006; Klein 1991; Wittmer et al. 2005; Zhong et al. 2008). Because of its important economic and ecological value, reindeer has recently been collected in "The List of Territorial Wild Life of National Protected Beneficial or Valuable Species for Economic and Scientific Research" stipulated by National Forestry Bureau of China, and considered to be one of the most worthy protected species.

In recent years, with the rapid development of molecular biological marker technology, the types of the marker technology have greatly increased. Compared with other genetic markers, in particular, microsatellite markers are more widely used. However, to the best of our knowledge, few genetic researches on reindeer of China have been studied. This is the first attempt to systematically understand the genetic variability of reindeer in China. The combined results of the study may provide scientific theoretical basis and reference on genetic diversity and resources for the protection and management of reindeer populations in China.

MATERIALS AND METHODS

Samples

A total of 163 fecal samples were collected from reindeer in the Greater Khingan Mountains of Inner Mongolia in China (Fig. 1). These were taken from 8 hunter points of the Using-reindeer Tribe (Table 1). The fecal samples were collected from December 25, 2013 to May 2, 2014 using the tracking method with the help of the local Ewenki people and reindeer-holders. In order to avoid sampling from the same individual, we numbered the reindeer in advance, we can differentiate individual reindeer by coloured ribbons tied on the neck of reindeer by Ewenki families combined with the other characteristics such

Fig. 1. Distribution of *Rangifer tarandus* sampled from eight geographic regions in China. The eight reindeer populations are Yang Shuanghu hunter points (YSH), Budongxia hunter points (BDX), Balajieyi hunter points (BLJY), Damala hunter points (DML), Dawa hunter points (DW), Guwenqiang hunter points (GWQ), Suoyulan hunter points (SYL) and Maliyasuo hunter points (MLYS).

as body size. After the reindeer defecated, we collected the fecal pellets in a ziplock bag using disposable plastic gloves, and recorded details of the individual's serial number and the date of sampling upon it. The average temperature of the region was at below -20°C, so samples could be stored temporarily at outside and stored in a -20°C freezer until analysis.

DNA preparation

Total genomic DNA was extracted from fecal samples of reindeer using the QIAamp DNA Stool Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions with some optimizations. An UV-visible spectrophotometer (NanoDrop 2000c, Thermo Scientific, USA) was used to detect the content and purity of the extracted DNA. Extracts were frozen at -20°C.

Microsatellite genotyping

Eleven high polymorphic microsatellite loci were selected from twenty-six microsatellite markers pre-chosen from red deer, goat, cattle and sheep. The markers were NVHRT01 and NVHRT31 (Røed and Hjell 1998), RT1, RT5, RT9, RT24 and RT25 (Wilson et al. 1997), T123, T156 and C217 (Jones et al. 2002), BM203 (Bishop et al. 1994). For analyzing fragment on Applied Biosystems 3100 Genetic Analyzers, the forward primers were endlabelled with one of three fluorescent dyes (FAM, TAMARA or HEX) (Invitrogen Shanghai Sangon Biological Engineer Technology & Services, Shanghai, China). We repeated PCR three times for each fecal sample to obtain reliable genotypes. Primer sequences and PCR conditions are shown in table 2.

We performed PCR amplifications in 10 μL reaction mixtures, comprising approximately 500 ng of template DNA, 1 μL 10*PCR Buffer, 0.8 μL dNTP Mixture, 0.2-1 mol of each primer, and 0.25U of *TaKaRa Taq* DNA polymerase (TaKaRa, Japan). Amplifications were performed using the following PCR procedure: an initial denaturation step for 5 min at 94°C, followed by 40 cycles of 94°C for 30 s, 30 s at locus-specific annealing temperature (51°C-57°C) and 1 min at 72°C, and a final elongation for 10 min at 72°C. The PCRs were performed on an Applied Biosystems Verti 96 well thermal. For genotyping, the PCR amplification products were separated by capillary electrophoresis.

Statistical analyses

Alleles were scored using GeneMapper version 3.2 (Applied Biosystems). Micro-Checker (van Oosterhout et al. 2004) was used to test the probability of the occurrence of null alleles and scoring error. The observed number of alleles (A), effective number of alleles (Ae), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (PIC), genetic distance (D), genetic identity (I) (Nei 1972), and statistical significance of Hardy-Weinberg equilibrium (HWE) were tested for by using Cervus 3.0.7 (Kalinowski et al. 2007), Genepop 4.0 software (Raymond and Rousset 1995), and Popgene version 1.31 (Yeh et al. 1999). ARLEQUIN 3.11 was taken to compute the AMOVA analyses (Excoffier et al. 2005). Wright's F-statistics was calculated using FSTAT version 2.9.3 (Goudet 2001). Using the unweighted pair group with the arithmetic mean method (UPGMA), a phylogenetic tree was constructed by MEGA 5.05 (Tamura et al. 2011).

Table 1. Information on the fecal samples of the reindeer

Name and abbreviation of hunter points	No. of samples	Longitude and latitude	Sampling year
Yang Shuanghu hunter points (YSH)	24	N51°41.490', E122°15.257'	2013
Budongxia hunter points (BDX)	27	N51°00.273', E121°24.609'	2013
Balajieyi hunter points (BLJY)	28	N51°41.593', E121°38.217'	2013
Damala hunter points (DML)	21	N51°16.026', E121°17.135'	2013
Dawa hunter points (DW)	27	N51°30.008', E121°42.105'	2014
Guwenqiang hunter points (GWQ)	20	N50°51.089', E121°51.612'	2013
Suoyulan hunter points (SYL)	7	N50°40.217', E121°01.387'	2014
Maliyasuo hunter points (MLYS)	9	N51°51.358', E122°31.612'	2013
Total	163		

RESULTS

Genetic diversity

The eleven microsatellite loci showed sufficient polymorphism for analyzing the genetic diversity of *Rangifer tarandus*. Null alleles were not detected from each locus by Micro-Checker. A total of 85 alleles were detected at the 11 microsatellite loci in 163 individuals of the 8 reindeer populations in China, and the average number of alleles per locus was 7.7, with the range of 4 (NVHRT31) to 11 (RT1). The values of H_O ranged from 0.2346 to 0.7143, with a mean of 0.4464. The values of H_E ranged from 0.6314 to 0.8523, with a mean of 0.7378. The average observed heterozygosity was less than the expected heterozygosity respectively (0.4464 ± 0.1565 and 0.7378 ± 0.0840). The values of PIC ranged from 0.566 to 0.836, with a mean of 0.701 (Table 2).

The mean observed number of alleles (A) was different among populations, ranging from 4.0000 to 7.0909. Of all the populations, the YSH, DML and BDX populations were the most diverse populations. The observed heterozygosity (H_O) and expected heterozygosity (H_E) per population ranged from 0.3736 to 0.5299 and from 0.6491 to 0.7608, with all values of H_O being less than that of H_E. Polymorphism information contents (PIC) ranged from 0.5654 to 0.7144, with the average of 0.6440, and obviously, PIC > 0.5 in all populations (Table 3).

Of 88 population-loci cases (8 populations x 11 loci) tested by Hardy-Weinberg equilibrium (HWE), 25 (28.41%) were in HWE ($P > 0.05$), and 63 (71.59%) deviated from HWE ($P < 0.05$). All of the loci departed from HWE in the BDX population, and only NVHRT31 was in HWE in the YSH population. Several loci were detected under HWE in other 6 populations with the number of 4 in BLJY, 2 in DML, 2 in DW, 4 in GWQ, 7 in SYL, 5 in MLYS. The F_{IS} values displayed that almost all loci presented heterozygote deficiencies ($F_{IS} > 0$, Table 4).

Genetic differentiation

The genetic differentiation was detected among 8 populations by the fixation indices F_{ST} (Table 5). The F_{ST} values ranged from -0.0015 to 0.0747. On the basis of Wright (Wright 1978), we detected a low level of genetic differentiation

Table 2. Summary statistics of eleven loci for eight *Rangifer tarandus* populations

Locus (accession no.)	Primer sequences (5' to 3')	Ta (°C)	Product size (bp)	No. of alleles	H_O	H_E	PIC
NVHRT01 (AF068203)	F-GCAGTCTTCCCCTTTCTT R-GATTGCAGAGTTGGACACTA	53	187-207	6	0.3025	0.7763	0.741
NVHRT31 (AF068211)	F-CATCCCAAAGTTTACAGCAG R-TTTTTATGGCCTGAGTGACAC	51	127-141	4	0.4136	0.6314	0.566
RT1 (U90737)	F-TGCCTTCTTTCATCCAACAA R-CATCTTCCCATCCTCTTTAC	52	198-238	11	0.7143	0.8523	0.836
RT5 (U90738)	F-CAGCATAATTCTGACAAGTG R-AATTCCATGAACAGAGGAG	54	136-168	10	0.5312	0.8335	0.812
RT9 (U90741)	F-TGAAGTTTAATTTCCACTCT R-CAGTCACTTTCATCCCACAT	51	109-131	9	0.6135	0.8394	0.818
RT24 (U90746)	F-TGTATCCATCTGGAAGATTTCAG R-CAGTTTAACCAGTCCTCTGTG	52	204-228	10	0.6478	0.7980	0.769
RT25 (U90747)	F-TGCCAAGGAACCAAGATGTC R-CATTCCAGTATTATTGCCTGA	57	186-210	7	0.3395	0.6923	0.657
T123 (AF192395)	F-GTTTCCTTGGCACATCTCT R-CTGTCGTTGTTGTTCTGTTG	56	119-143	6	0.3312	0.6932	0.654
T156 (AF192396)	F-TCTTCCTGACCTGTGTCTTG R-GATGAATACCCAGTCTTGTCTG	56	162-218	8	0.3889	0.7034	0.653
C217 (AF102242)	F-GCAGGAAGGAGGAGACAGTA R-GCTGGTTCGTTATCATTTAGC	56	164-192	5	0.3937	0.6546	0.597
BM203 (G18500)	F-GGGTGTGACATTTTGTTCCC R-CTGCTCGCCACTAGTCCTTC	56	187-217	9	0.2346	0.6411	0.611
Average				7.7	0.4464	0.7378	0.701

H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content.

among all populations generally, except between populations YSH and DW (0.0747), BDX and SYL (0.0525), BLJY and SYL (0.0662), DW and SYL (0.0623), which was a moderate level of genetic differentiation ($0.05 < F_{ST} < 0.15$). The most genetically distant populations were YSH and DW populations (0.0747), while no significant genetic differentiation was detected between BDX and MLYS populations (-0.0015). AMOVA test in 8 reindeer populations based on 11 microsatellite loci showed 4.11% genetic variation among populations and 95.89% of variation was attributed to among individuals within populations (Table 6).

Table 3. Genetic diversity of the eight *Rangifer tarandus* populations based on eleven microsatellite loci

Populations	A	Ae	H_O	H_E	PIC
YSH	7.0909	4.5938	0.4606	0.7608	0.7144
BDX	6.5455	3.9907	0.3736	0.7301	0.6803
BLJY	6.0909	3.0569	0.4554	0.6491	0.5873
DML	6.3636	4.0232	0.4577	0.7456	0.6861
DW	6.0909	3.6154	0.3978	0.6821	0.6235
GWQ	5.2727	3.5257	0.5299	0.7136	0.6524
SYL	4.0000	2.8161	0.4675	0.6663	0.5654
MLYS	4.8182	3.6024	0.5152	0.7308	0.6427
Mean	5.7841	3.6530	0.4572	0.7098	0.6440

A, observed number of alleles; Ae, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content.

Table 4. *P* value of chi-square test for Hardy-Weinberg equilibrium and F_{IS}

Locus	YSH		BDX		BLJY		DML		DW		GWQ		SYL		MLYS	
	P	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}
NVHRT01	0.0000*	0.359	0.0000*	0.788	0.0000*	0.773	0.0055*	0.529	0.0000*	0.675	0.0087*	0.329	0.0001*	0.833	0.0380*	0.603
NVHRT31	0.2127	0.231	0.0000*	0.711	0.0000*	0.130	0.0064*	0.586	0.3827	0.513	0.0002*	0.163	0.4976	0.280	0.0235*	0.443
RT1	0.0004*	0.364	0.0000*	0.115	0.2372	0.010	0.3870	0.073	0.0003*	0.235	0.0702	-0.034	0.1344	0.360	0.0672	0.164
RT5	0.0005*	0.279	0.0000*	0.392	0.0000*	0.384	0.0000*	0.354	0.0000*	0.445	0.0000*	0.198	0.1600	0.419	0.3859	0.403
RT9	0.0034*	0.342	0.0000*	0.447	0.8189	0.214	0.0166*	0.458	0.0000*	0.226	0.0456*	0.291	0.0482*	0.333	0.0417*	0.207
RT24	0.0015*	0.237	0.0000*	0.230	0.1444	0.212	0.1352	0.417	0.0033*	0.449	0.4398	0.072	0.5828	0.040	0.1566	0.232
RT25	0.0000*	0.614	0.0000*	0.551	0.0000*	0.680	0.0000*	0.423	0.0042*	0.449	0.1024	0.351	0.1116	0.273	0.8646	-0.157
T123	0.0000*	0.467	0.0000*	0.548	0.0000*	0.456	0.0076*	0.651	0.0000*	0.423	0.0010*	0.684	0.0347*	0.486	0.0167*	0.453
T156	0.0000*	0.569	0.0001*	0.564	0.0196*	0.374	0.0000*	0.661	0.0000*	0.725	0.0507	0.261	0.0088*	0.500	0.0321*	0.355
C217	0.0000*	0.357	0.0000*	0.700	0.0817	0.371	0.0060*	0.261	0.1994	0.174	0.0000*	0.619	0.2547	0.016	0.0005*	0.467
BM203	0.0000*	0.481	0.0000*	0.802	0.0000*	0.622	0.0000*	0.666	0.0000*	0.940	0.0000*	0.580	0.2281	0.769	0.1061	0.860
Mean		0.3909		0.5316		0.3842		0.4617		0.4776		0.3195		0.3917		0.3664

F_{IS} = inbreeding coefficient; *P* = probability of significant deviation from Hardy-Weinberg equilibrium; $^*P \leq 0.05$.

Table 5. Pairwise F_{ST} (below the diagonal) of eight populations of *Rangifer tarandus* in China

Populations	YSH	BDX	BLJY	DML	DW	GWQ	SYL	MLYS
YSH								
BDX	0.0364							
BLJY	0.0442	0.0118						
DML	0.0326	0.0028	0.0141					
DW	0.0747	0.0330	0.0412	0.0202				
GWQ	0.0215	0.0373	0.0427	0.0238	0.0430			
SYL	0.0373	0.0525	0.0662	0.0351	0.0623	0.0180		
MLYS	0.0226	-0.0015	0.0069	0.0056	0.0197	0.0083	0.0124	

F_{ST}, F-statistics of genetic differentiation coefficient among populations.

Population genetic relationships

The Nei unbiased genetic distances (Nei 1972) between each two different populations range from 0.0800 to 0.3107, whereas the genetic identity ranged from 0.7329 to 0.9231 (Table 7). The farthest genetic distance was found between populations BDX and SYL, and populations BDX and BLJY had the nearest genetic distance. Cluster analysis performed using UPGMA divided the eight populations into two groups (Fig. 2). The first group was population SYL, and the second group included the remaining seven populations. In the second group, the relationship between populations BDX and BLJY was closest, population DML was clustered with BDX and BLJY and then clustered with populations DW and MLYS. Populations YSH and GWQ were also close. These five populations (BDX, BLJY, DML, DW and MLYS) were clustered together and then clustered

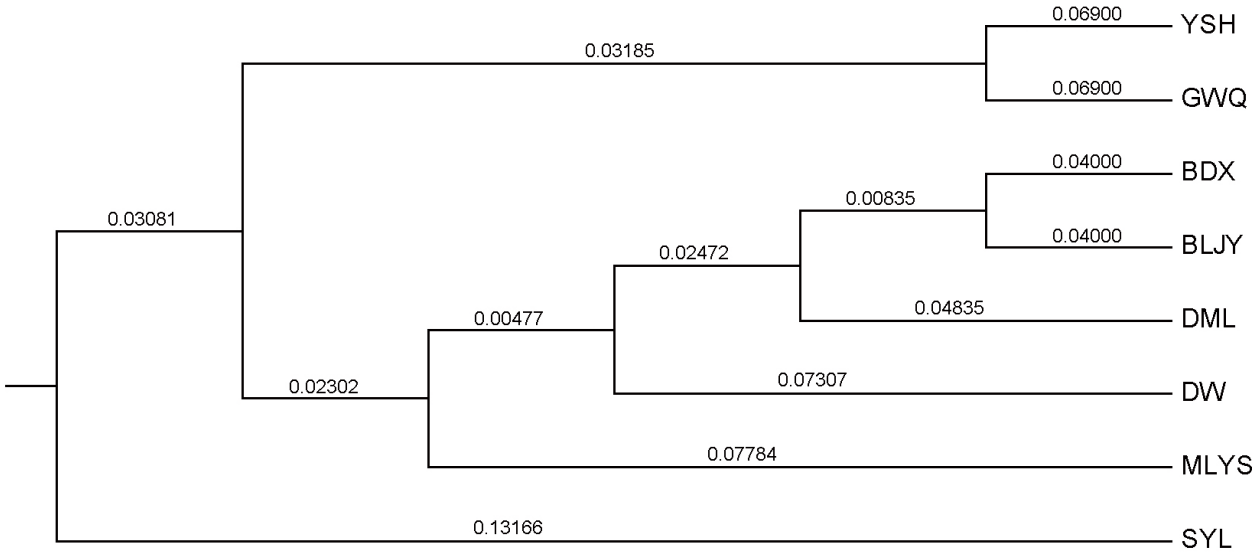

Fig. 2. Cluster analysis result among eight reindeer populations. Unweighted pair group method with arithmetic mean (UPGMA) in MEGA software were used for this analysis.

Table 6. Analysis of molecular variance (AMOVA) for 8 reindeer populations based on 11 microsatellite loci

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation (%)	Significance tests (*P*)
Among populations	7	77.238	0.17438	4.11	<0.01
Within populations	318	1294.581	4.07101	95.89	<0.01
Total	325	1371.819	4.24539	100	<0.01

Table 7. Inter-population genetic identification (up triangle) and genetic distances (down triangle) between 8 geographic populations

Population	YSH	BDX	BLJY	DML	DW	GWQ	SYL	MLYS
YSH		0.8078	0.8121	0.8460	0.7673	0.8711	0.7508	0.7497
BDX	0.2135		0.9231	0.9085	0.8754	0.8377	0.7329	0.8783
BLJY	0.2082	0.0800		0.9072	0.8512	0.8369	0.7344	0.8667
DML	0.1672	0.0960	0.0974		0.8657	0.8481	0.7764	0.8324
DW	0.2649	0.1331	0.1611	0.1442		0.8328	0.7525	0.8465
GWQ	0.1380	0.1770	0.1780	0.1647	0.1829		0.8425	0.8415
SYL	0.2866	0.3107	0.3087	0.2531	0.2844	0.1713		0.7957
MLYS	0.2881	0.1297	0.1430	0.1834	0.1666	0.1726	0.2285	

with populations YSH and GWQ.

DISCUSSION

Genetic diversity is an important basis of germplasm resources assessment, and high genetic diversity indicates good survival capability and breeding potential (O'Connell and Wright 1997). In the present study, we aimed to increase the information of genetic diversity and the differentiation among 8 reindeer (*Rangifer tarandus*) populations in China. For this purpose, the eleven microsatellite markers, which were chosen from twenty-six loci, were successfully amplified to detect genetic variation sampled from 8 reindeer populations in China. In this study, the number of alleles over 11 microsatellite loci ranged from 4 (NVHRT31) to 11 (RT1). The average number of alleles per locus was 7.7, which was higher than the 2.4 alleles per locus detected by Côté et al. (2002) and the 2.0-6.6 alleles per locus detected by Cronin et al. (2003), and lower than the 10.78 alleles per locus of the domestic reindeer in Alaska (Mager et al. 2013, 2014). In our study, we found that the value of A of SYL population was lowest among all populations (A = 4.0000), suggesting that SYL population may have experienced population declines recently. However, the number of samples of SYL population was less than that of other populations, which may be the cause of the difference. Moreover, it is worth noting that there is a clear gap between A (mean 5.7841) and Ae (mean 3.6530) for all populations, which suggests an imbalance in the distribution of alleles in the populations studied.

The average level of population heterozygosity reflects the degree of genetic consistency in a given populations. Cronin et al. (2003) reported that the average observed heterozygosity for 19 caribou and reindeer herds ranged from 0.33 to 0.50, which was lower than the average expected heterozygosity (0.417-0.480). It is reported that the average expected heterozygosity for four caribou herds (n = 245) and reindeer (n = 67) ranged from 0.62 to 0.86 using 19 microsatellite loci (Colson et al. 2014; Mager et al. 2013; 2014). In the present study, the heterozygosity of each population was approximate to 0.5, indicating a moderate level of genetic diversity. GWQ and MLYS population had a higher heterozygosity of all populations, while a lower level of that occurs on BDX and DW population. If a population is under HWE, the value of H_O is close to H_E, and the population deviates from HWE as a result of the excess and/or deficit of heterozygotes reflected by the inbreeding coefficient (F_{IS}) (Quan et al. 2006). And fixation index (F_{IS}) was used as a measure of heterozygote deficiency or excess (Wright 1978). However, all values of H_O are lower than H_E in all reindeer populations, and heterozygote deficiency seems to be the strongest evidence for all these deviations from HWE. The heterozygote deficiency could be explained as a Wahlund effect if population subdivision is occurring, linkage with loci under selection (genetic hitchhiking), population heterogeneity, null alleles or inbreeding. However, the inbreeding coefficiency (F_{IS}) observed in the populations ranged from 0.3195 (GWQ population) to 0.5316 (BDX population). Positive F_{IS} values suggested inbreeding to be the main causes for shortage of heterozygotes in the reindeer populations. It was reported that inbreeding has lowered the viability of the reindeer to levels far below those of the 1950s, with many calves suffering from congenital abnormalities (Li 1988). Indeed, the mean population sizes in China are not large enough to prevent inbreeding. Moreover, the habitat fragmentation and extensive management, made the gene exchange between populations unlikely, are also the factors of inbreeding. Such phenomena are warning us to pay enough attention to germplasm conservation, otherwise it might be a potential risk for *Rangifer tarandus* to rapidly lose genetic diversity and suffer further inbreeding.

The value of F_{ST}, an estimator of genetic differentiation between these populations, was defined by Wright (1978). And it is generally assumed that $F_{ST} < 0.05$, $0.05 < F_{ST} < 0.15$, $0.15 < F_{ST} < 0.25$ and $F_{ST} > 0.25$ exhibit no, moderate, significant, and high levels of genetic diversity, respectively (Wright 1978). In this study, F_{ST} values of most pairs were significantly below 0.05, indicating an extremely low level of genetic differentiation among all populations generally. The result was consistent with AMOVA test that the differentiation was very weak among the eight populations. Interestingly, there was no genetic diversity between BDX and MLYS populations (F_{ST} = -0.0015), suggesting that they may be some sort of communication including reindeer exchange before. Cluster analysis performed using UPGMA based on genetic distances demonstrated that the relationship between the eight populations is not completely consistent with their geographic. Reindeer in China originated from Siberia,

arrived in the Greater Khingan Mountains in northeast China in 1654. They have lost the habit of migration along with the semi-domestication. There are currently 8 small isolated populations of reindeer with the management of the Ewenki peoples, separated from each other by natural forest barriers and human activities. In addition, the Ewenki peoples rarely exchange their reindeer. Relaxed polygyny early on could have a significant impact on diversity since the loss of diversity is greatest when the population size is small and polygyny further reduces the effective number of breeders.

In the future, the protection and managements of reindeer in China should be strengthened. A useful tactic would be to increase their current exchange rate among the 8 reindeer populations, and introduce some reindeer into the existing populations from other relevant countries. In addition, the related ecological compensation policy should be implemented to incentivize reindeer herding, considering all reindeer in China are managed by Ewenki peoples. An economic and protective management plan should be developed. Meanwhile, establishing natural reserve may be one of the most important and effective approaches for the in-site conservation of reindeer in China.

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