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Genetic Diversity Estimation of Six Goat Populations from Chongqing, China using Microsatellite Markers

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ABSTRACT

Goats are an important domestic animal for animal husbandry that has attracted considerable research attention worldwide. Genetic diversity assessment and population genetic structure of indigenous goats can provide valuable conservation strategies. The current study aimed to evaluate the genetic diversity and population structure of six goat populations from Chongqing, China. A total of 145 animals from five local goat populations, namely, Youzhou black (UZG), Jianzhou (JZG), Banjiao (BJG), Dazu (DZG) and Hechuan white goat (HCG) and an introduced breed called Nubia goat (NBG) located in Chongqing, China were genotyped with 18 autosomal microsatellite markers. A series of genetic diversity parameters and population phylogeny were estimated and constructed. This study preliminarily showed that the six goat populations present rich genetic diversity and significant genetic divergence among them. Furthermore, material exchanges were observed between the introduced and local goat populations in Chongqing. This result will further explain the diversity conservation of Chongqing goat populations.

Key words: Genetic diversity, Genetic structure, Goat, Microsatellite marker.

Domestication of goats first began 10,000 years ago (Zeder and Hesse, 2000; Kumar *et al.,* 2018; Bertolini *et al.,* 2018). After goats spread worldwide from domestication centers, the phenotypic characteristics of different local population and population have huge differences because of the differences in the ecological environment, culture in habitat regions and artificial breeding of human with breeding requirements (Guang-Xin *et al*., 2018; Wang *et al*., 2017).

More than 90% of goats are distributed in Asia and Africa (Yang *et al*., 2018). However, the large number of high-productivity introduced breeds has led to a decline in the population size of native populations and extensive gene flow between commercial breeds and local populations has affected the conversion of local goat populations (Guang-Xin *et al*., 2018; Yang *et al*., 2021). Microsatellite markers in eukaryotes (Yadav *et al*., 2015) have been widely used to investigate the genetic diversity of a variety of domestic animals, such as deer (Yang *et al*., 2018), pig (Kharzinova and Zinovieva, 2020) and chicken (Habimana *et al*., 2020).

This study aimed to explore the genetic diversity and population genetic structure relationships of six goat populations (five local populations and one introduced breed) in Chongqing, China using 18 autosomal microsatellite markers to provide a data reference for their conservation.

Six goat populations, including five indigenous populations (Table 1), namely, Banjiao (BJG, 21), Dazu black (DZG, 24), Hechuan white (HCG, 24), Jianzhou big-ear (JZG, 30) and Youzhou Wu (UZG, 25) goats and an introduced breed called Nubian goat (NBG, 21). A total of 145 samples with unrelated kinship were randomly collected. Venous blood (5 mL) was collected from each goat and genomic DNA was extracted using the Steypure Universal Genomic DNA Extraction Kit (Chongqing, China).

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Eighteen microsatellite markers (INRA023, ILSTS005, INRABERN185, MAF065, INRA063, ILSTS011, OarFCB20, SRCRSP7, ILSTS029, SPS113, CSRD247, SRCRSP5, SRCRSP8, SRCRSP9, TCRVB6, MAF70, OarFCB48 and TGLA53) were used for genotyping of samples and genetic diversity analysis similar to a previous study (Guang-Xin *et al*., 2020). Primers of microsatellite markers were synthesized by Wuhan Tianyi Huiyuan Biological Co., Ltd.

A 15 µL system, including 0.35 µL of upstream and downstream primers (10 µmol/L), 1 µL of DNA (~100 ng/µL), 7.5 µL of 2×Accurate Taq master mix (Accurate Biology, Chongqing) and 5.8 µL of ddH_2O, was used for PCR amplification. The following program of the PCR amplification system was applied: predenaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 51°C-61°C for 30 s, extension at 72°C for 30 s and 30 cycles. Finally, amplification was extended at 72°C for 5 min and products were stored at 4°C. PCR products were genotyped using the ABI3730 platform (AB, US).

Observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphic information content (PIC) and mean

number of alleles (N_A) were analyzed using the microsatellite toolkit (Freeman *et al.,* 2004). Hardy-Weinberg equilibrium (HWE) and genetic differentiation coefficient (F_{ST}) were calculated using Arlequin (Excoffier *et al*., 2007). Inbreeding coefficients within population (FIS) were analyzed using FSTAT software (Goudet, 1995). PHYLIP software package (Felsenstein, 1989) was used to construct a phylogenetic tree on the basis of Nei's genetic distance and iTOL (https://itol.embl.de/) online website was utilized for visualization. STRUCTURE 2.3.3 (Pritchard *et al*., 2000) was applied to perform Bayesian population structure clustering analysis with 100 repetitions and STRUCTURE_harvester (http://taylor0.biology.ucla.edu/struct_harvest/) online tool was used to assess the optimal K value. Furthermore, the structural result was visualized using CLUMP (Jakobsson and Rosenberg, 2007) and DISSTRUCT 1.1 (Rosenberg, 2004).

A total of 271 alleles were detected in all samples (Table 1) and N_A of each microsatellite marker ranged from 8 (MAF70) to 28 (INRA023). H_E ranged from 0.58 (SRCRSP7 and ILSTS029) to 0.92 (CSRD247) and H_O ranged from 0.22 (ILSTS005) to 0.81 (MAF065), with an average value of 0.58. PIC of each marker ranged from 0.56 (SRCRSP7 and ILSTS029) to 0.91 (CSRD247), with an average value of 0.74.

The results were higher than those of Indian (Yadav *et al.,* 2015), Brazilian (Menezes *et al*., 2020) and Chinese goat breed populations along the Yangtze River (Guang-Xin *et al*., 2018) despite their similarity with Chinese dairy (Wang *et al*., 2017) and endangered Spanish (Serrano *et al*., 2009) goat breeds. Therefore, the six goat populations in Chongqing demonstrated rich genetic diversity.

The HWE analysis results of all markers (Table 2) showed that 11 markers deviate from HWD in the JZG population while BJG deviates from HWE with the minimum number of markers (4). Notably, ILSTS005 deviated from HWE within all six populations while INRA063, OutFCB20 and OutFCB48 did not deviate from HWE among the populations.

The genetic diversity of population demonstrated that N_A of the six goat populations ranges from 5.83±1.98 (UZG) to 8.22±2.44 (JZG), PIC ranges from 0.56 (UZG) to 0.67 (JZG), H_E ranges from 0.62±0.042 (UZG) to 0.72±0.029 (JZG) and H_O ranges from 0.52±0.024 (JZG) to 0.64±0.024

Table 1: Genetic diversity estimation of 18 microsatellite markers among six goat populations.

Marker	H_E	H_O	PIC	N_A
INRA023	0.90	0.68	0.89	28
ILSTS005	0.71	0.22	0.68	10
INRABERN185	0.64	0.43	0.62	12
MAF065	0.83	0.81	0.80	14
INRA063	0.86	0.74	0.84	12
ILSTS011	0.69	0.54	0.67	16
OarFCB20	0.70	0.69	0.67	14
SRCRSP7	0.58	0.29	0.56	10
ILSTS029	0.58	0.35	0.56	17
SPS113	0.71	0.55	0.66	10
CSRD247	0.92	0.66	0.91	18
SRCRSP5	0.86	0.73	0.84	19
SRCRSP8	0.80	0.52	0.77	19
SRCRSP9	0.84	0.68	0.82	18
TCRVB6	0.83	0.69	0.81	19
MAF70	0.72	0.60	0.68	8
OarFCB48	0.75	0.65	0.72	13
TGLA53	0.82	0.68	0.79	14
Mean	0.76	0.58	0.74	15.06

Table 2: Hardy Weinberg equilibrium analysis of six goat populations at 18 microsatellite markers.

Marker	Population					
	BJG	DZG	HCG	JZG	NBG	UZG
INRA023	0.28766	0.00285	0.42691	0.00789	0.49067	0.04256
ILSTS005	0.00008	0.00000	0.00019	0.00000	0.01258	0.00948
INRABERN185	1.00000	0.40251	0.02683	0.00021	0.00417	0.33689
MAF065	0.57544	0.21199	0.99318	0.10164	0.0035	0.55269
INRA063	0.30974	0.5746	0.68395	0.12848	0.10226	0.83559
ILSTS011	0.71154	0.02682	1.00000	0.01751	0.99308	0.08601
OarFCB20	0.06124	0.48033	0.62746	0.54187	0.97269	0.10739
SRCRSP7	0.33844	0.53621	0.03176	0.00000	0.03802	0.00292
ILSTS029	1.00000	0.18917	0.00000	0.01879	0.58202	1.00000
SPS113	0.20178	0.00095	0.00502	0.06655	0.00118	0.00299
CSRD247	0.00330	0.00107	0.17419	0.0042	0.00526	0.31071
SRCRSP5	0.07428	0.01304	0.2176	0.00483	0.79534	0.76495
SRCRSP8	0.00728	0.28647	0.02342	0.00043	0.10128	0.22588
SRCRSP9	0.20064	0.34958	0.00656	0.00000	0.00002	0.00000
TCRVB6	0.38814	0.09758	0.18185	0.07513	0.93101	0.02208
MAF70	0.29980	0.0176	0.2221	0.00018	0.77357	0.38871
OarFCB48	0.56624	0.14641	0.70933	0.26131	0.35823	0.13386
TGLA53	0.03040	0.78140	0.92006	0.05514	0.84902	0.09264

(NBG). Notably, F_{IS} of each population ranged from 0.043 (NBG) to 0.284 (JZG) and F_{IS} of all populations was statistically insignificant (P>0.00046, Table 3). Although the above-mentioned populations were not yet under inbred, the results of HWE analysis indicated that the conservative status of their genetic diversity should be monitored.

In particular, H_E of the six goat populations was higher than their H_O in this study, thereby indicating that decreased heterozygosis is common in these goat populations. Notably, the small population size and unbalanced mating ratio, particularly when a few satisfactory breeding males provided the group a high proportion of spouse rights, will cause the population to deviate from the balance and lead to increased risks in the population (Yang *et al*., 2021; Guang-Xin *et al*., 2019; Basang *et al*., 2021). Therefore, some of these populations, especially JZG and BJG, still showed potential risks of inbreeding.

Subsequently, the result of F_{ST} analysis showed that the F_{ST} pair among populations is significant (P<0.05), thereby indicating a large and significant genetic divergence among populations (Table 4). The smallest difference pair (F_{ST}=0.0479, P<0.05) was identified between DZG and HCG, whereas the largest difference pair (F_{ST}=0.23306, P<0.05) was identified between HCG and JZG.

The phylogenetic results (Fig 1) showed that the three populations, namely, NBG, UZG and BJG, cluster into one; HCG and DZG cluster together and JZG clusters individually. Except for the introduced breed (NBG), population phylogenetic relationships of other local populations were consistent with their geographical distribution of habitats.

The results of population structure (Fig 2) analysis in this study showed that DZG and HCG are initially separated from all populations when K=2, JZG populations are separated from the remaining four populations to form a cluster when K=3 and the BJG population is separated into another cluster when K=4. In addition, K=3 obtained the optimal K value (Table 5) and the population structure pattern was consistent with previous reports (Guang-Xin *et al*., 2015).

The geographical environment of Chongqing is complex because several criss-crossing mountains and rivers that serve as a natural geographical barrier for wild and domestic animal populations affect the exchange of genetic material among them (Yang *et al*., 2021; Yuan *et al*., 2012). The phylogenetic relationship and population clustering of populations, especially HCG and DZG, were in accordance with their sampling locations.

Notably, Youyang (the UZG sample location) is far from Kaizhou; however, UZG and the two goat populations from Kaizhou (NBG and BJG) are grouped together. Hence, the phylogenetic relationship of Chongqing indigenous populations fails to match completely with their geographical distribution. This phenomenon is widely observed in domestic animals (Guang-Xin *et al.,* 2018). Therefore, extensive historical exchanges of genetic material are

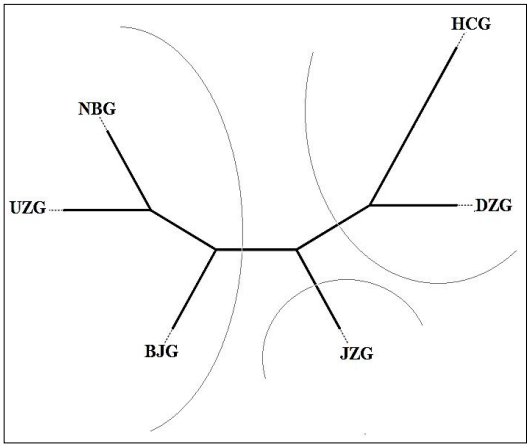


Fig 1: Phylogenetic network of six goat populations using Nei's genetics distance.

Table 3: Genetic diversity estimation of six goat populations using 18 microsatellite markers.

Population	H_E	H_O	PIC	N_A	F_{IS}	*P*-value
BJG	0.65±0.049	0.56±0.025	0.59	5.94±1.80	0.129	0.0005
DZG	0.67±0.036	0.62±0.025	0.61	5.94±2.69	0.079	0.0037
HCG	0.64±0.036	0.60±0.025	0.58	6.17±2.43	0.053	0.0509
JZG	0.72±0.029	0.52±0.024	0.67	8.22±2.44	0.284	0.0005
NBG	0.66±0.044	0.64±0.024	0.61	6.56±2.62	0.043	0.0755
UZG	0.62±0.042	0.58±0.025	0.56	5.83±1.98	0.059	0.0296

Table 4: Pair-wise difference (F_{ST}) among six goat populations using 18 microsatellite markers.

	BJG	DZG	HCG	JZG	NBG	UZG
BJG	-					
DZG	0.1551*	-				
HCG	0.1993*	0.0479*	-			
JZG	0.1829*	0.2013*	0.2306*	-		
NBG	0.0798*	0.1442*	0.1967*	0.1551*	-	
UZG	0.0946*	0.1748*	0.2076*	0.18029*	0.0507*	-

*Significant at P<0.05.

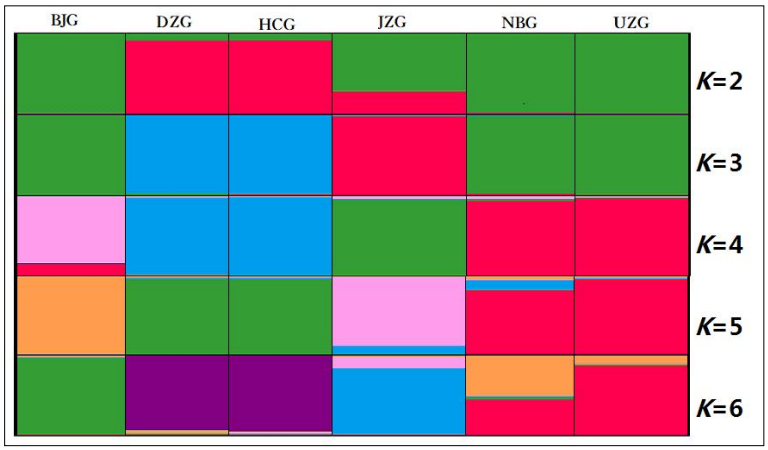


Fig 2: Population structure clustering of six Chongqing goat populations using 18 microsatellite markers.

Table 5: Most credible K value estimation of STRUCTURE result.

K	Reps	Mean LnP (K)	Stdev LnP (K)	Ln' (K)	[Ln"(K)]	Delta K
2	100	-8070.365000	29.291238	-	-	-
3	100	-7405.355000	1.746418	665.001000	551.866000	315.998861
4	100	-7292.220000	835.456906	113.135000	3.266000	0.003909
5	100	-7182.351000	705.520434	109.889000	136.931000	0.194085
6	100	-7209.413000	265.961343	-27.062000	-	-

observed among original goat populations after introducing NBG. In particular, human migration and trade activities cause genes to flow easily among adjacent animal population habitats.

CONCLUSION

The phylogenetic relationship and genetic diversity of six goat populations were identified in this study using microsatellite markers. The results showed that Chongqing goats present a rich level of genetic diversity and the absence of inbreeding. In addition, we observed an exchange of genetic material not only among populations with adjacent habitats but also those with large geographical distances through phylogenetic tree analysis and STRUCTURE clustering. This finding may be related to human migration and business activities.

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