



Association between Microsatellite Polymorphism and Body Size Traits in Sonid Bactrian Camels

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ABSTRACT

In this study, 17 microsatellite markers were used to analyze the genetic diversity of Sonid bactrian camels, and the correlation between microsatellite markers and their body size was analyzed to find microsatellite markers associated with the body size traits of Sonid bactrian camels, so as to provide reference for marker-assisted selection of Sonid bactrian camels. The results showed that 64 alleles were detected in 17 microsatellite markers. The average number of alleles was 3.7647, the average heterozygosity was 0.6205, and the average polymorphism information content was 0.5602. It showed that the population of Sonid bactrian camel had high genetic diversity. Among 17 microsatellite markers, 4 microsatellites were found to be associated with body size traits of Sonid bactrian camels. The body length of AB genotype labeled LCA33 was significantly higher than that of AA genotype ($P < 0.05$), and the chest circumference, tube circumference and body weight of AB genotype were significantly higher than those of AA genotype and AC genotype ($P < 0.05$). The chest circumference and body weight of AA genotype marked LCA90 were significantly higher than those of AC genotype and BB genotype ($P < 0.05$). The body length of AB genotype marked CMS36 was significantly higher than that of AA genotype and BB genotype ($P < 0.05$). The body weight of CE genotype marked YWLL44 was significantly higher than that of AC genotype, BD genotype and BE genotype ($P < 0.05$), the circumference of CE genotype was significantly higher than that of AC genotype ($P < 0.05$), and the chest circumference of CE genotype was significantly higher than that of AC genotype and BD genotype ($P < 0.05$). The other 13 microsatellite markers had no significant effect on the body size of Sonid bactrian camels.

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Authors' Contribution

JYB conceived and designed the study and conducted the lab work. RTDW and QZ analyzed the data and wrote the article. DB and LMD helped in sampling. XHT helped in analysis of data.

Key words

Sonid bactrian camel, Microsatellite markers, Genetic diversity, Body size traits, Association analysis

INTRODUCTION

Bactrian camels, which are special livestock species with double back humps and tall body and adapting to desert and semi-desert regions, mainly live in deserts, and their range of activity is mainly located in western and northern Asia. The region where bactrian camels live is very narrow and it is only restricted to arid areas, so they are precious livestock species in the world. In recent years, domestic and foreign researches regarding hair characteristics (Wuren *et al.*, 2017), dairy products (Zhang *et al.*, 2016), blood physiological properties (Bai *et al.*, 2015), genetic diversity (Hedayat-Evrigh *et al.*, 2018; Banerjee *et al.*, 2012) and organization structure (Ye *et al.*, 2014a, 2014b) of bactrian camels have achieved progress.

Microsatellite markers have been extensively applied to genetic diversity studies of cow (Ni *et al.*, 2018), sheep (Bai *et al.*, 2015) and poultry (Bai *et al.*, 2016a, 2016b, 2016c, 2017) by virtue of high abundance,

good repeatability, co-dominance marker and selective neutrality. It is also widely used in plant genetic diversity research (Yang *et al.*, 2013; Li *et al.*, 2017; Guo *et al.*, 2018). Genetic diversity of 17 microsatellite markers in the Sonid Bactrian camel population was analyzed in this study, association analysis of these microsatellite markers with body weight and body size and other traits was conducted, and microsatellite markers associated with body size traits of Sonid bactrian camels was found out so as to provide a reference for marker assisted selection, protection and rational utilization of Sonid bactrian camels.

MATERIALS AND METHODS

Test material

Random collection of 40 Sonid bactrian camels, blood was taken from jugular vein (10ml from each bactrian camel), ACD anticoagulant was added for anticoagulation, blood DNA was extracted using the whole-blood genomic DNA extraction kit method from Beijing Dingguo Changsheng, and it was preserved under -20°C . Seventeen microsatellite markers with high polymorphism were screened (Evdotchenko *et al.*, 2003; Prasad *et al.*, 2014),

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Table I. Microsatellite marker information.

Name	Primer sequence	Fragment size (bp)	Annealing temperature
CVRL101	F:GAAGAGGTTGGGGCACTAC; R:CAGGCAGATATCCATTGAA	188-253	60
CMS15	F:AAAACCTAAAGCCAGAAAGGCCAAA; R:TTTTCCAGATCTTGACCAC	81-121	58
CMS18	F:GAACGACCCTTGAAGACGAA; R:AGCAGCTGGTTTTAGGTCCA	144-166	55
CMS36	F:TGCTTTCCAGTTGTTTGCTG; R:GCAAGGTGGTGTGGAGATT	195-227	55
CMS104	F:CACTTAGGTCCCTGGGCTTT; R:GCATTCTCTGCATCGTGTG	75-101	55
LCA33	F:GAGCACAGGGAAGGATATTCA; R:ACAGCAAAGTGATTCCATAATACA	122-167	55
LCA37	F:TAATTACCTCCCCACCACA; R:TGGACCCAGGACTTGAAATG	143-183	55
LCA63	F:TTACCCAGTCCTTCGTGGG; R:GGAACCTCGTGGTTATGGAA	213-233	54
LCA66	F:GTGCAGCGTCCAAATAGTCA; R:CCAGCATCGTCCAGTATTCA	212-241	55
LCA71	F:CCGTATCTATCTATACACACACACA; R:TCACCCTCCTCTATTTGG	127-167	55
LCA82	F:CGTGACACCAGGCTAAGTGA; R:TTTCAGATGGTAGCTTAAAAATTG	85-129	55
LCA90	F:TATAACCCTGGTCTCGCCAA; R:CCAAGTAGTATTCCATTATGCG	237-248	55
VOLP08	F:CCATTCACCCCATCTCTC; R:TCGCCAGTGACCTTATTTAGA	142~172	53.3
VOLP32	F:GTGATCGGAATGGCTTGAAA; R:CAGCGAGCACCTGAAAGAA	147~203	53.3
YWLL29	F:GAAGGCAGGAGAAAAGGTAG; R:CAGAGGCTTAATAACTTGCAG	200-228	55
YWLL36	F:CAAATGTTGTAATAATTGCACAGAAC; R:TTCAGGTTTGCCTAGTTTAACTGT	149-189	55
YWLL44	F:CTCAACAATGCTAGACCTTGG; R:GAGAACACAGGCTGGTGAATA	95-111	55

and the sequence of primers was shown in Table I. The primers were synthesized by Shanghai Shengggong Bioengineering Technology Service Co., Ltd.

PCR amplification

Pre-denaturation at 94 for 4 min, then denaturation at 94 for 40 s, annealing at 60 for 1 min, annealing at 72 for 20 seconds, denaturation, annealing and elongation were carried out for 35 cycles, then elongation at 72 and finally the reaction was completed and cooled and preserved at 4 C.

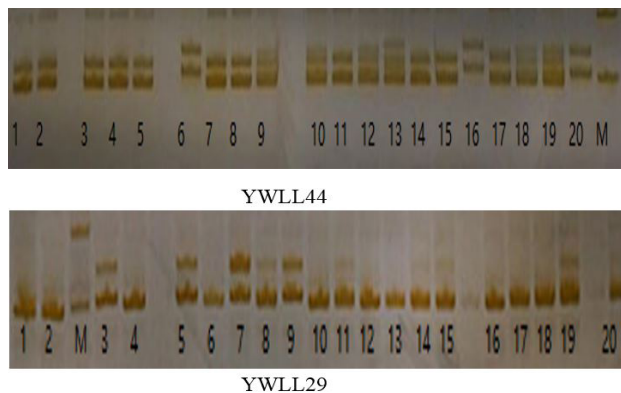


Fig. 1. Polymorphism of microsatellite markers YWLL44 and YWLL29 in *Sonid bactrian camels*.

Table II. Allele frequencies of microsatellite markers.

Microsatellite markers	Alleles				
	A	B	C	D	E
LCA33	0.6000	0.0875	0.2500	0.0625	
LCA37	0.4750	0.5000	0.0250		
LCA63	0.4250	0.1375	0.3625	0.0750	
LCA66	0.1625	0.3375	0.1625	0.3375	
LCA71	0.0625	0.4625	0.4750		
LCA82	0.4500	0.0500	0.4500	0.0500	
LCA90	0.3500	0.2750	0.1750	0.2000	
CMS15	0.0375	0.2875	0.4625	0.2125	
CMS18	0.5000	0.2875	0.1875	0.0250	
CMS36	0.5875	0.4125			
CMS104	0.5125	0.3750	0.1125		
CVRL101	0.1375	0.3000	0.2000	0.2375	0.1250
YWLL29	0.6625	0.1125	0.2250		
YWLL36		0.0250	0.4750	0.0250	0.4750
YWLL44	0.0250	0.4375	0.0625	0.2625	0.2125
VOLP08	0.2250	0.2750	0.2250	0.2750	
VOLP32	0.2875	0.2125	0.2875	0.2125	

The amplified product 5 μ l was detected by 2% agarose electrophoresis at 120 V for 30 min. After electrophoresis, the results were observed under ultraviolet light and photographed on the basis of DNA marker D2000.

Table III. Polymorphic information of microsatellite markers.

Microsatellite markers	Na	Ne	O_Hom	O_Het	E_Hom	E_Het	Ave_Het	PIC
LCA33	4.0000	2.3038	0.2500	0.7500	0.4269	0.5731	0.5356	0.5111
LCA37	3.0000	2.0997	0.0000	1.0000	0.4696	0.5304	0.5248	0.4103
LCA63	4.0000	2.9712	0.0000	1.0000	0.3282	0.6718	0.6756	0.6004
LCA66	4.0000	3.5635	0.0000	1.0000	0.2715	0.7285	0.7330	0.6680
LCA71	3.0000	2.2551	0.0500	0.9500	0.4364	0.5636	0.5727	0.4566
LCA82	4.0000	2.4390	0.0000	1.0000	0.4025	0.5975	0.6045	0.5039
LCA90	4.0000	3.7209	0.2500	0.7500	0.2595	0.7405	0.7270	0.6823
CMS15	4.0000	2.9144	0.0000	1.0000	0.3348	0.6652	0.6782	0.5938
CMS18	4.0000	2.7142	0.0000	1.0000	0.3604	0.6396	0.6292	0.5664
CMS36	2.0000	1.9406	0.8750	0.1250	0.5092	0.4908	0.3795	0.3672
CMS104	3.0000	2.4042	0.1250	0.8750	0.4085	0.5915	0.5728	0.5000
CVRL101	5.0000	4.5262	0.0000	1.0000	0.2111	0.7889	0.7464	0.7437
YWLL29	3.0000	1.9913	0.5500	0.4500	0.4959	0.5041	0.4025	0.4410
YWLL36	4.0000	2.2099	0.0000	1.0000	0.4456	0.5544	0.5993	0.4446
YWLL44	5.0000	3.2258	0.0000	1.0000	0.3013	0.6987	0.6963	0.6373
VOLP08	4.0000	3.9604	0.0000	1.0000	0.2430	0.7570	0.7413	0.7003
VOLP32	4.0000	3.9120	0.0000	1.0000	0.2462	0.7538	0.7298	0.6968
Mean	3.7647	2.8913	0.1235	0.8765	0.3618	0.6382	0.6205	0.5602

SSCP

15% non denaturing polyacrylamide gels were used to detect the products, silver nitrate dyeing method is used for dyeing, mainly through fixation, oxidation, dyeing, color rendering, photography and other links.

Statistical analysis

Popgene32 software was used to calculate numbers of effective alleles, allele frequencies and heterozygosity of microsatellites. SPSS software was used to conduct association analysis of microsatellites with body weight, and the following was the analytical model: $y_{ijkl} = \mu + S_i + M_j + G_k + e_{ijkl}$, where y_{ijkl} is trait phenotypic value, μ is ensemble average and S_i is the i (th) sex effect; M_j is the j (th) age effect; G_k is the k (th) genotype effect; e_{ijkl} is residual error effect.

RESULTS AND DISCUSSION

Polymorphism detection of microsatellite markers

The polyacrylamide gel electrophoresis results of PCR products of some markers are presented in Figure 1. The allele frequencies of microsatellite markers in Sonid bactrian camel population are shown in Table II. The maximum number of alleles detected by CVRL101

is 5, the minimum number of alleles detected by CMS36 is 2, the number of alleles detected by other markers is between 3 and 4.

The polymorphism of microsatellite markers can be seen in Table III, 64 alleles were detected in 17 microsatellite markers. The average number of alleles was 3.7647, the average heterozygosity was 0.6205, and the average polymorphism information content was 0.5602. It can be seen that the Sonid bactrian camel has high genetic diversity. The study carried out by Gao *et al.* (2009) showed that the average polymorphic information content value of microsatellite markers detected in hundreds of bactrian camels from 13 areas of China and Mongolia was 0.5414. Tian *et al.* (2012) studied the genetic diversity of bactrian camels from 6 places of Xinjiang by microsatellite markers, and showed that all of the used microsatellite markers presented high polymorphism, with polymorphic information content values ranging from 0.6099 to 0.6551. Vijn *et al.* (2007) conducted a microsatellite marker analysis of 4 dromedary species in India, and results indicated that a large number of alleles were under a low frequency. So the polymorphic level of microsatellite markers in this study was moderate, intermediate between Gao *et al.* (2009) and Tian *et al.* (2012).

Table IV. Association between microsatellite markers and body size of Sonid bactrian camels.

Name	Genotype	Body height/cm	Body length/cm	Bust/cm	Round tube/cm	Weight/ kg
LCA33	AA	161.000±0.0330 ^a	139.6000±2.9257 ^b	201.6000±3.3373 ^b	16.8000±0.2494 ^b	445.2900±17.6812 ^b
	AB	168.2000±2.7459 ^a	154.0000±3.3015 ^a	225.4000±4.2614 ^a	18.4000±0.5099 ^a	568.9090±22.5901 ^a
	AC	163.3889±2.2854 ^a	145.8889±2.5112 ^{ab}	211.4444±4.2994 ^{ab}	17.2778±0.3110 ^{ab}	498.6865±22.2293 ^{ab}
	AD	165.8000±0.9695 ^a	146.4000±2.5416 ^{ab}	218.6000±5.9883 ^{ab}	17.2000±0.3741 ^{ab}	521.3083±26.7245 ^{ab}
	BC	157.5000±7.5000 ^a	141.0000±9.0000 ^{ab}	198.5000±6.5000 ^b	16.5000±0.5000 ^b	437.5177±41.8083 ^b
LCA37	AB	163.2105±1.3303 ^a	144.8947±1.6611 ^a	210.8421±2.7096 ^a	17.2895±0.1919 ^a	493.0276±13.8334 ^a
	BC	167.0000±2.0000 ^a	150.0000±4.0000 ^a	213.5000±2.5000 ^a	16.5000±0.5000 ^a	510.1657±1.2928 ^a
LCA63	AB	164.0000±2.7928 ^a	148.0000±6.2928 ^a	210.6000±7.0964 ^a	17.8000±0.6633 ^a	500.1861±40.2844 ^a
	AC	163.4483±1.5604 ^a	144.9655±1.7281 ^a	212.0690±3.0681 ^a	17.1379±0.2089 ^a	497.0368±15.3422 ^a
	BD	162.6667±3.5839 ^a	143.6667±4.7935 ^a	206.0000±7.1039 ^a	17.3333±0.4944 ^a	473.3970±37.8698 ^a
LCA66	AC	165.8462±2.6234 ^a	145.1538±2.9566 ^a	213.5385±5.8677 ^a	17.4615±0.3859 ^a	505.7870±29.4103 ^a
	BD	162.2222±1.3788 ^a	145.1481±1.9235 ^a	209.7407±2.6287 ^a	17.1481±0.2046 ^a	488.1536±13.7101 ^a
LCA71	AC	161.8000±4.1641 ^a	141.4000±3.6551 ^a	202.8000±5.7740 ^a	16.8000±0.3741 ^a	453.7243±27.2104 ^a
	BB	162.0000±5.0000 ^a	142.5000±1.5000 ^a	208.0000±5.0000 ^a	17.0000±0.0000 ^a	471.2891±13.4620 ^a
	B	163.7273±1.4123 ^a	145.8788±1.8431 ^a	212.3939±2.9538 ^a	17.3333±0.2161 ^a	501.3388±15.1859 ^a
LCA82	AC	162.9167±1.2359 ^a	145.1944±1.6382 ^a	210.6944±2.5054 ^a	17.2222±0.1695 ^a	492.1925±12.4821 ^a
	BD	167.7500±6.5494 ^a	144.7500±6.9447 ^a	213.5000±14.1745 ^a	17.5000±1.1902 ^a	509.1126±77.6066 ^a
LCA90	AA	166.0000±1.4960 ^a	150.1429±2.9230 ^a	221.5714±5.0749 ^a	17.8571±0.5084 ^a	544.7257a±27.4807 ^a
	AC	163.7857±1.6811 ^a	144.6429±2.4147 ^a	210.5000±3.4668 ^b	17.2857±0.1941 ^a	488.7325±17.2819 ^b
	BB	163.6667±8.6474 ^a	146.6667±9.8375 ^a	207.0000±9.0185 ^b	17.6667±0.8819 ^a	483.2152±52.1286 ^b
	BD	161.8750±2.3977 ^a	143.1250±2.6690 ^a	207.5000±4.8140 ^b	16.8750±0.3275 ^a	478.1499±24.5348 ^b
CMS15	AB	165.3333±2.6666 ^a	146.3333±4.1766 ^a	218.3333±11.2891 ^a	17.0000±0.5773 ^a	522.6098±51.1865 ^a
	BC	163.6000±1.5481 ^a	144.6500±1.7877 ^a	210.2000±3.1217 ^a	17.1500±0.1956 ^a	487.9703±14.9405 ^a
	CD	162.8235±2.3890 ^a	145.5294±3.1096 ^a	210.5882±4.5794 ^a	17.4118±0.3644 ^a	495.7731±24.6920 ^a
CMS18	AB	164.0000±1.7286 ^a	144.5652±1.9819 ^a	213.1739±3.6930 ^a	17.2609±0.2608 ^a	500.9518±18.9254 ^a
	AC	162.0000±2.1224 ^a	145.4667±3.0282 ^a	207.2000±3.8436 ^a	17.1333±0.2905 ^a	480.7806±20.0080 ^a
	AD	167.0000±1.0000 ^a	149.5000±0.5000 ^a	214.0000±1.0000 ^a	18.0000±0.0000 ^a	510.8888±2.3896 ^a
CMS36	AA	162.7619±1.6314 ^a	144.5714±1.9130 ^b	211.7143±3.4751 ^a	17.2381±0.2478 ^a	494.6097±17.3440 ^a
	AB	167.4000±1.8055 ^a	154.0000±4.3243 ^a	214.6000±3.0757 ^a	17.2000±0.3741 ^a	525.4455±16.1662 ^a
	BB	162.9286±2.6195 ^a	142.8571±2.9442 ^b	208.5714±5.2043 ^a	17.2857±0.3695 ^a	481.5249±26.8202 ^a
CMS104	AA	166.0000±3.7859 ^a	151.6667±6.1191 ^a	210.6667±4.7022 ^a	17.0000±0.5773 ^a	505.8133±33.0667 ^a
	AB	163.4615±1.7639 ^a	144.2308±2.0140 ^a	211.8077±3.6089 ^a	17.3077±0.2403 ^a	495.8371±18.4025 ^a
	AC	162.0000±2.2173 ^a	143.6667±2.9154 ^a	208.3333±4.7900 ^a	17.1111±0.4231 ^a	478.9616±22.6887 ^a
	BB	165.0000±3.0000 ^a	154.0000±9.0000 ^a	212.5000±2.5000 ^a	17.5000±0.5000 ^a	517.7604 ±33.1562 ^a
CVRL101	AB	165.6000±1.0295 ^a	144.2000±1.5937 ^a	217.0000±5.9916 ^a	17.0000±0.0000 ^a	509.7066±26.4570 ^a
	AC	163.1667±1.7966 ^a	142.0000±4.0166 ^a	207.6667±7.1305 ^a	17.0000±0.4472 ^a	473.8557±34.8770 ^a
	BD	162.8421±1.5844 ^a	146.3158±2.2530 ^a	211.6842±3.3113 ^a	17.3684±0.2560 ^a	498.6778±17.5233 ^a
	CE	163.5000±4.1048 ^a	145.3000±4.2165 ^a	208.6000±6.7019 ^a	17.3000±0.5174 ^a	488.8834±34.9227 ^a
	AA	164.2727±1.7434 ^a	146.2273±2.1370 ^a	211.2727±3.5144 ^a	17.2273±0.2626 ^a	497.7660±18.5661 ^a
YWLL29	AC	161.0000±2.7487 ^a	141.6667±2.7436 ^a	209.1111±5.7019 ^a	17.1111±0.2605 ^a	477.8839±26.1093 ^a
	BC	163.6667±2.6510 ^a	146.0000±4.0276 ^a	212.1111±5.5987 ^a	17.4444±0.4746 ^a	500.3969±28.4474 ^a
	BD	156.0000±9.0000 ^a	137.5000±8.5000 ^a	202.5000±13.5000 ^b	16.0000±0.0000 ^a	444.7873±64.0855 ^a
YWLL36	CE	163.7895±1.2639 ^a	145.5526±1.6207 ^a	211.4211±2.6445 ^a	17.3158±0.1889 ^a	496.4685±13.5022 ^a
	AC	164.0000±5.0000 ^a	144.0000±10.0000 ^a	205.5000±5.5000 ^b	16.5000±0.5000 ^b	467.9481±43.5104 ^b
YWLL44	BD	162.5714±1.8510 ^a	144.6190±2.2860 ^a	208.4762±3.2038 ^b	17.0476±0.2233 ^{ab}	482.9272±16.3426 ^b
	BE	162.5000±1.5468 ^a	144.6429±2.4798 ^a	212.5714±4.4987 ^{ab}	17.4286±0.2911 ^{ab}	498.0232±21.9750 ^b
	CE	173.0000±7.0237 ^a	152.0000±7.0000 ^a	224.6667±15.8359 ^a	18.3333±1.3333 ^a	568.5623±86.5378 ^a
	AC	161.0000±1.8185 ^a	142.6111±2.2825 ^a	206.5556±3.8866 ^a	17.0556±0.2056 ^a	471.9134±18.9111 ^a
	BD	165.3636±1.6922 ^a	147.2273±2.1601 ^a	214.5909±3.3183 ^a	17.4091±0.2917 ^a	511.8608±17.6782 ^a
VOLP08	AC	164.6087±1.8657 ^a	145.8261±2.2419 ^a	209.4348±3.3242 ^a	17.4348±0.2654 ^a	490.2826±17.8966 ^a
	BD	161.7647±1.5846 ^a	144.2353±2.2630 ^a	213.0588±4.1253 ^a	17.0000±0.2425 ^a	498.7577±19.8198 ^a

Association between polymorphism of microsatellite markers and body size traits of bactrian camels

The association between microsatellite marker polymorphism and body size traits of bactrian camels is shown in Table IV. From Table IV, it can be seen that the body length of AB genotype marked LCA33 is significantly higher than that of AA genotype ($P<0.05$), and the chest circumference, tube circumference and body weight of AB genotype marked LCA33 are significantly higher than those of AA genotype and AC genotype ($P<0.05$). The chest circumference and body weight of A A genotype marked LCA90 were significantly higher than those of AC genotype and BB genotype ($P<0.05$). The body length of AB genotype marked CMS36 was significantly higher than that of AA genotype and BB genotype ($P<0.05$). The body weight of CE genotype marked YWLL44 was significantly higher than that of AC genotype, BD genotype and BE genotype ($P<0.05$), the tube circumference of CE genotype marked YWLL44 was significantly higher than that of AC genotype ($P<0.05$), and the chest circumference of CE genotype marked YWLL44 was significantly higher than that of AC genotype and BD genotype ($P<0.05$). The other 13 microsatellite markers had no significant effect on the size traits of Sonid bactrian camels ($P>0.05$). Therefore, microsatellite markers LCA33, CMS36, LCA90 and YWLL44 can be used in assistant selection of body size and weight markers for Sonid bactrian camels. Which was similar to the study results of Wuren *et al.* (2018a, 2018b). By now, very few studies on bactrian camels association analysis and genetic mapping were reported, which limited the superior alleles exploring and marker assisted selection breeding developing. The 4 microsatellite markers associated with body size or body weight traits in this study would be valuable in improving bactrian camels by molecular polymerization breeding, which could gather all of the superior alleles from 4 microsatellite markers into one individual.

There are more than 280,000 camels in China, of which Inner Mongolia Autonomous Region is the largest, accounting for about 67% of the total number of camels in China, followed by Xinjiang Uygur Autonomous Region, accounting for about 20%. To protect the genetic diversity of bactrian camel resources in Inner Mongolia Autonomous Region is not only to rationally manage and utilize the existing resources, but also to maintain a certain resource potential for future needs.

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Statement of conflict of interest

The authors declare there is no conflict of interest.

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