Original

Development of an effective microsatellite marker system to determine the genetic structure of *Meriones meridianus* populations

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Abstract: Understanding the genetic quality of the gerbil, *Meriones meridianus,* plays an important role in the study of medical biology. However, no effective system has been established for evaluating a population's genetic diversity to date. In the present study, we established a set of reasonable evaluative systems based on microsatellite markers of the Mongolian gerbil by using the method of cross-amplification of species. Following electrophoresis analysis, short tandem repeat (STR) scanning, and sequencing, 11 microsatellite loci were identified by matching the criteria characteristics and were used to evaluate the genetic diversity of two stocks of *Meriones meridianus: Meriones meridianus jei* Wang, 1964 (*M. m. jei*) and *Meriones meridianus cryptorhinus* Blanford, 1875 (*M. m. cryptorhinus*) from Xinjiang, China. The microsatellite loci screened were highly polymorphic and were suitable for genetic quality control of *Meriones meridianus*. In addition, the quality of the non-bred *M. m. jei* and *M. m. cryptorhinus* strains in our study is sufficient for them to be promising stocks in the future for the farmed animal industry.
Key words: biomarker, genetic diversity, *Meriones meridianus*, microsatellite loci

Introduction

The gerbil, *Meriones meridianus* (Rodentia, Muridae, Gerbillinae), otherwise known as the midday gerbil, belongs to Rodentia, Cricetidae, *Meriones*, is native to the sandy deserts in Afghanistan, China, Iran, Azerbaijan, Kazakhstan, Kyrgyzstan, Mongolia, Russia, Tajikistan, Turkmenistan, and Uzbekistan [26]. It also widely distributed in the Inner Mongolia Xinjiang Plateau in China.

Meriones meridianus can serve as an animal model for biological and medical research in many aspects. For several decades, *Meriones meridianus* has been used as an animal model for research, human medicines, and animal anti-disease breeding, such as studies of echino-coccosis [21], viral hepatitis [39], the sensibility of plague virus [31], a hantavirus infection model [38], a helicobacter infection model [9], and the duration and characteristics of the course of cutaneous leishmaniasis [27]. *Meriones meridianus* has also benefited a great deal of research on ethology [30].

So far, the major physiological and biochemical indices, including reproductive performance, reproductive biology [32], structure of parasympathetic ganglia [13], and thermogenesis parameters [2], have been systematically evaluated for *Meriones meridianus* populations. In order to breed better varieties of this species, two stocks, *Meriones meridianus jei* Wang, 1964 (*M. m. jei*) and *Meriones meridianus cryptorhinus* Blanford, 1875 (*M. m. cryptorhinus*), were captured in Turpan and Lun-

tai areas of Xinjiang, china, in June 1992 [35]. Currently, forty-three generations of these stocks have been bred, and biochemical information of the stocks has been estimated from their urine [37]. The *Meriones meridianus* individuals used throughout this study were originated from the fortieth generations.

The genetic quality of *Meriones meridianus* has an important influence on the accuracy, repeatability, and scientificity of medical biological research results. If genetic quality standards have not been established, it is difficult to monitor the genetic quality within each stock and unify the quality among a population, and this has become one of the obstacles for widespread monitoring of genetic quality. While some basic research on the genetic diversity of *Meriones meridianus* has been conducted using molecular biology methods [10], to this day, there are still no suitable standards for genetic evaluation of *Meriones meridianus*.

Many methods are utilized to monitor genetic variation, such as biochemical marker gene detection, immunological trait marker gene detection, and DNA polymorphism. Among them, simple sequence repeats (SSRs) or microsatellites (MS) are extremely valuable tools for assessing population genetic diversity because of their distinctive features [29].

Microsatellites are characterized by short tandem repeats (STRs) of 1 to 6 nucleotide motifs, which widely spread in a random manner throughout the eukaryotic genome. They are commonly used in a wide range of applications, such as the evaluation of genetic diversity and population structure patterns [3, 16]. In previous studies, microsatellites have also been used as biomarkers for monitoring heritable traits in rodents [7, 33]. Because the whole genome of *Meriones meridianus* has not been sequenced completely, it is difficult to obtain suitable microsatellite markers by genome sequencing.

It has been reported that cross-species amplification of species microsatellite loci in related species is an effective way to isolate microsatellite markers [14, 15, 22, 24]. Since the midday gerbils and Mongolian gerbils are the closely related species, the present study aimed to identify microsatellite loci for *Meriones meridianus* on the basis of a significant microsatellite marker system (28 loci markers) using the method of cross-amplification of species, which has already been established for the Mongolian gerbil [8]. On the basis of this research, we discuss the genetic structure of the *Meriones meridianus* population in the Xinjiang area of China.

Material and Methods

Ethics

All of the experimental procedures were conducted in accordance with the Guidelines of the Capital Medical University Animal Experiments and Experimental Animals Management Committee (No.AEEI-2017-032).

Sample background and collection

All the samples were available from the Center for Laboratory Animal Research of Xinjiang district in China. Forty *Meriones meridianus* subspecies (20 *M. m. jei* and 20 *M. m. cryptorhinus*; 1.5 months old; half male and half female; weight, 80–100 g and 60-80g respectively) were captured in the Turpan and Luntai areas of Xinjiang in June 1992. The locations of the sampled gerbil populations chosen as the original gerbils to be bred are shown in Fig. 1. *Meriones meridianus* individuals were bred at 17–26°C with a relative humidity of 30–65% and were placed in an experimental animal room with visible light and darkness for 12 h, respectively. *Meriones meridianus* individuals captured in the wild will likely fight if housed together, causing injury
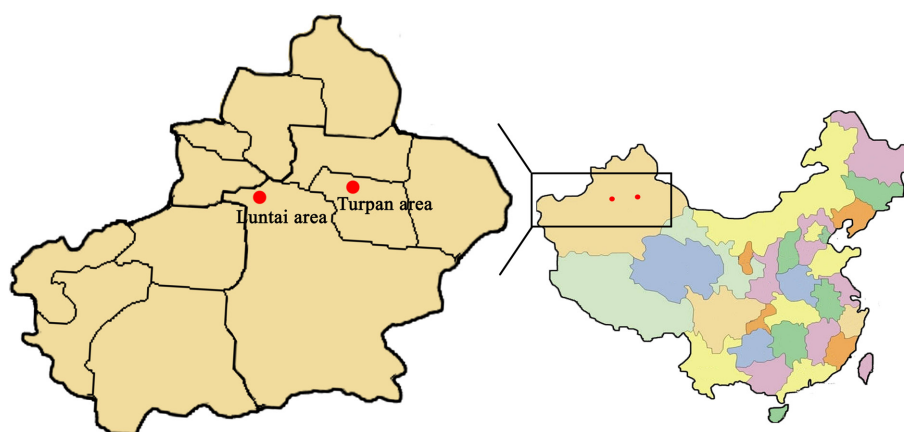

Fig. 1. Maps of the *Meriones meridianus* populations showing the sample locations in the Turpan and Luntai areas in Xinjiang, China.

or death; therefore, one mature female and one mature male or two mature females and one mature male were selected for pairing. Pairings were determined by observation of several hours without fighting. The number of litters ranged from 1 to 8, and the average ratio of males to females was 1.4:1.0. To date, 43 generations of gerbils have been bred.. The *Meriones meridianus* individuals used in this study originated from the fortieth generations. In total, 63 DNA samples were extracted from 39 *M. m. jei* and 24 *M. m. cryptorhinus* individuals and preserved in sterile tubes at −80°C.

DNA extraction

In order to develop microsatellite loci for *Meriones meridianus*, total genomic DNA was extracted from 63 tail tissue samples following a previously described method [6]. The concentration and purity of DNA from each sample were determined by A260/280 measurement using a microplate absorbance reader system (Bio-Rad, Hercules, CA, USA), and the sample quality was further evaluated by 0.5% (w/v) agarose gel electrophoresis. DNA was then diluted to 100 ng/μl and stored at −20°C for later use as polymerase chain reaction (PCR) templates.

PCR procedure

For each 20 μl PCR amplification, the following reagents were used: 2 μl 10X buffer, 1.0 μl each of 10 μM primer; 1.0 μl dNTPs (100 μM; Takara Co., Ltd., Tokyo, Japan), 0.2 μl Taq high-fidelity DNA polymerase (1.0U; Takara), and 1.0 μl template DNA (50–100 ng). Amplifications were performed in a gradient thermal cycler (ALS1296, Bio-Rad) using the following protocol: Predenaturation was performed at 94°C for 5 min, and then 35 cycles of denaturation were performed at 94°C for 30 s. The annealing temperature was set by a gradient PCR approach the temperature range of 45°C to 67°C. The optimum annealing temperature was selected, which allowed clear DNA bands to be distinguished in agarose gel. Extension was then performed at 72°C for 30 s, followed by a final extension at 72°C for 7 min. Amplified products were stored at 4°C for further analysis. The genotypes of 28 microsatellites loci for 63 *Meriones meridianus* individuals were detected using PCR with fluorescent primers, each labeled with a different commercially available dye label (FAM, HEX, TAMRA).

Agarose gel electrophoresis

Agarose was weighed and added into 0.5× TBE (Solarbio, Beijing, China) to make 2% gel, and ethidium bromide (EB) was added to a final concentration of 0.5 μg/ml. After the electrophoresis for 35 min at 120 V,

images of the agarose gel were observed by ultraviolet imager and analyzed.

Microsatellite loci selection

All scanned data were analyzed with Gene Scan 3.7. The results revealed that there are two types of species, those that were homozygous, which mainly display a single waveform, there were both homozygous individuals, which mainly had no polymorphic positions, and those that were heterozygous, which had polymorphic positions, among the animals studied. The polymorphic microsatellite loci were selected for cloning and sequencing by Ruibiotech Co. (Beijing, China). Finally, by searching PubMed, we identified the appropriate target sequence.

Analysis of population genetic structure

The population genetic diversity and genetic structure were assessed using 11 microsatellite loci. Alleles of loci were classified as A, B, C or D according to amplified fragments. Genotypes of each microsatellite locus in all samples were input into POPGENE 3.2 in the form of AB and BB to observe significant genetic structure indices, including the observed and effective numbers of alleles, Shannon information index, observed and effective heterozygosity, and polymorphic loci (Poly-loci), were calculated for genetic variation analysis within the populations. In addition, the genetic distance between *M. m. jei* and *M. m. cryptorhinus* was measured according to the method for Nei's standard distance [18].

Results

Selection of microsatellite loci

Previous studies have suggested that 28 microsatellite loci are optimal for identifying the Mongolian gerbil [8]. Since *Meriones meridianus* and the Mongolian gerbil both belong to the subfamily Gerbillinae, we can make use of the Mongolian gerbil microsatellite loci for filtering. The first round of selection was performed with 28 microsatellite loci of the Mongolian gerbil through PCR assays. After initial PCR assays, 19 primer sets generated the desired amplification products under modified conditions. Partial results of agarose gel electrophoresis are shown in Fig. 2, in which individual strips appear in pure individuals and two strips appear in hybrid individuals. Further STR screening, shown in Fig. 3, revealed that 13 loci matched microsatellite criteria. Primer sequences, annealing temperatures, range of allele genes, and GenBank accession numbers for the 13 microsatellites are shown in Table 1. To further verify the results obtained from STRscanning, we sent microsatellite loci
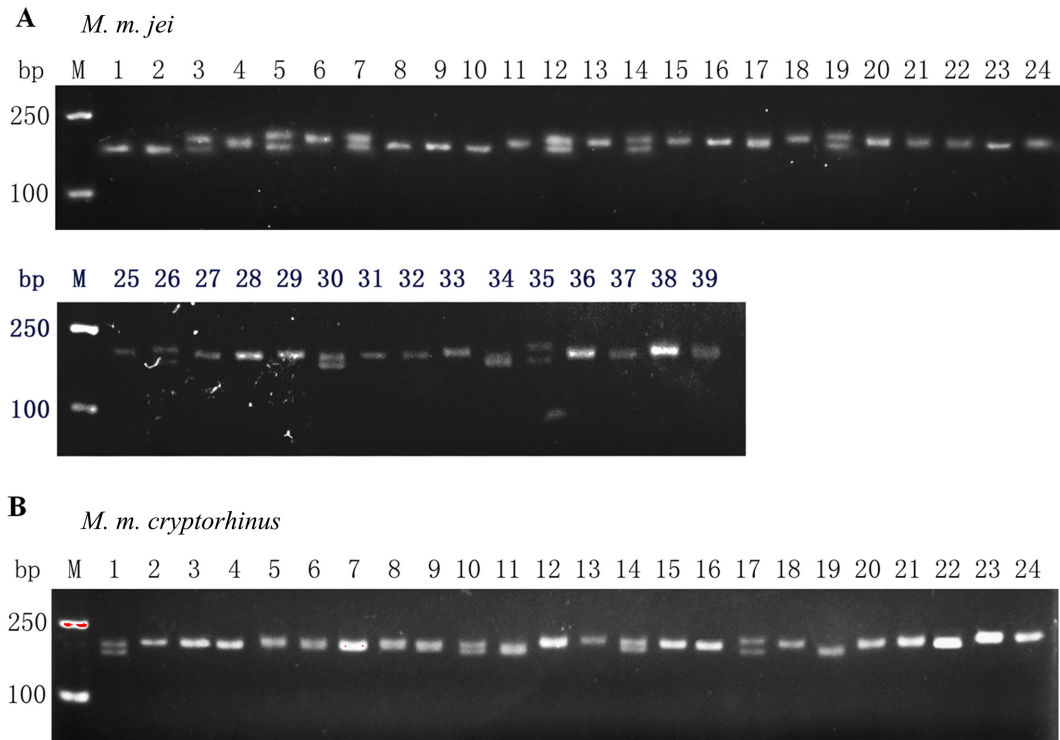
Fig. 2. PCR amplification of the gerbil genome by primers for microsatellite loci: *AF200943*, which showed heterogeneity and homogeneity for the *M. m. jei* (A) and *M. m. cryptorhinus* (B) populations of *Meriones meridianus* gerbils in this study.
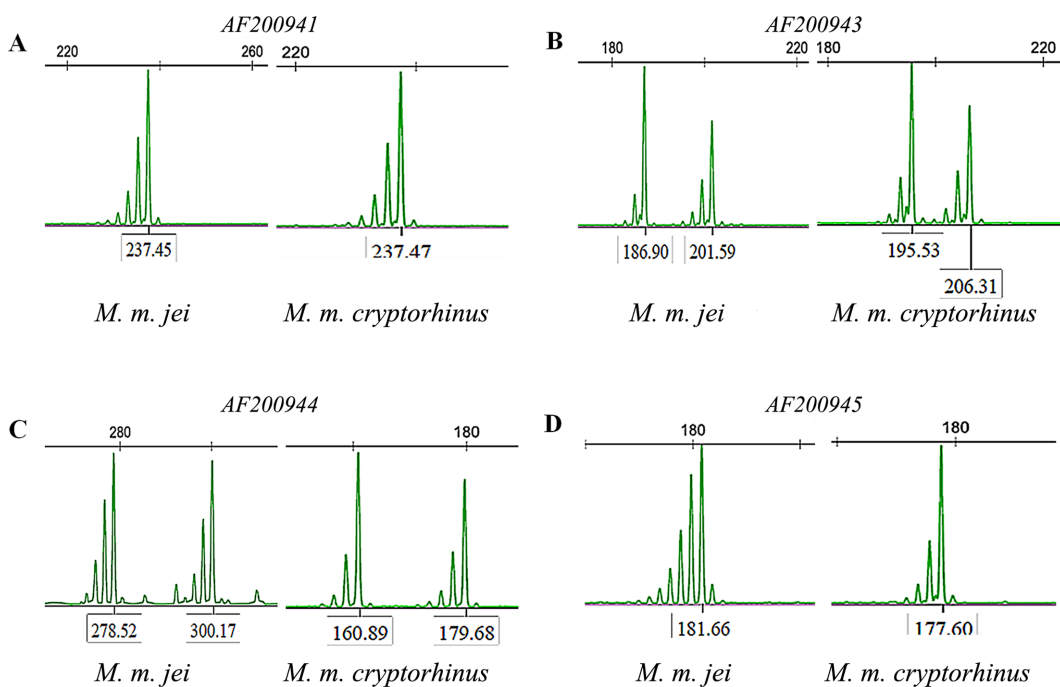

Fig. 3. STR loci *AF200941* (A), *AF20043* (B), *AF200944* (C), and *AF200945* (D) showed microsatellite polymorphisms in *Meriones meridianus* gerbils.

to Ruibiotech Co. (Beijing, China) to confirm the sequencing. The sequencing was performed using an ABI3730XL auto-sequencing machine system. Partial results of cloning and sequencing are shown in Fig. 4. With the exception of *D19Mit1* and *DXMit17*, all loci were polymorphic and showed different degrees of variability. *D19Mit1* and *DXMit17* were discarded, as they had one allele in *M. m. jei* and *M. m. cryptorhinus* population for further verification. As shown in Table 2, *AF200941*, *AF200942*, *AF200943*, *AF200944*,

Table 1. Characteristics of the 13 microsatellite loci isolated from *Meriones meridianus* gerbils

AN	Primer sequences(5'–3')	*M.m.jei*		*M.m.cryptorhinus*		Mg^{2+}(mM)	Chr
		AT(°C)	Rng(bp)	AT(°C)	Rng(bp)		
AF200941	TGGGTCCTTTGGAAGA TGGCTTAAAATGAATCACTTA	55°C	170–230	53.8°C	219–237	2	
AF200942	CAGGCACCCCCAGTTT GTCTACACAGGCTGAGGATGT	54°C	120–150	50.8°C	137–143	2	
AF200943	GGCTCCTGATTCTACATTTCT CAACCATTGGCAACTCTC	55°C	180–200	53.8°C	195–206	2	
AF200944	GCTGGGCTTTAATGTTTATTT GGTGGCTCACACTTTCTGT	55°C	200–300	57.5°C	158–179	2	
AF200945	AGTCCCTATTACATCCACAAG TTATCCTGCAAAGCCTAAG	58°C	150–180	50.8°C	160–177	2	
AF200947	GACAGAGTGGGAGGGGTATGT TGGCAAGTTTGGTTTGTTTGA	55°C	180–207	57.5°C	197–199	2	
GU562706	CAGGAATAAAGTATAATGGGGTGC CCCATGATCAGTTGGGTTTT	50°C	216–228	50.8°C	227	2	*D16Mit26*
GU562777	GCTCCCTTTCCTCTTGAACC GGGCCCTTATTCTATCTCCC	53°C	110–130	50°C	186–192	2	*D2Mit22*
GU562747	AACACATGAAACGTGTGCGT TGATAGGCATGCTTAAGCCC	50°C	230–245	48.3°C	243	2	*D3Mit130*
GU562700	AATCCTTGTTCACTCTATCAAGGC CATGAAGAGTCCAGTAGAAACCTC	50°C	157	50°C	157	2	*D19Mit1*
GU562704	CCTCTGAGGAGTAACCAAGCC CACAGAGTTCTACCTCCAACCC	50.8°C	210–235	50.8°C	228–236	2	*D17Mit38*
GU562695	CCTGTTTGGGCACCTAGATT TAATAACCCATGTTTTCTGTGGG	50°C	215	45.4°C	237	2	*DXMit17*
GU562725	ACACTCAGAGACCATGAGTACACC GAGTTCACTACCCACAAGTCTCC	53°C	135	50.8°C	111–127	2	*D8Mit56*

AN, GenBank accession No.; AT, optimized annealing temperature; Mg^{2+} (mM), concentration of Mg^{2+} (mM) for PCR; Chr, position on mouse chromosome of the corresponding mouse locus; Rng, allele range.
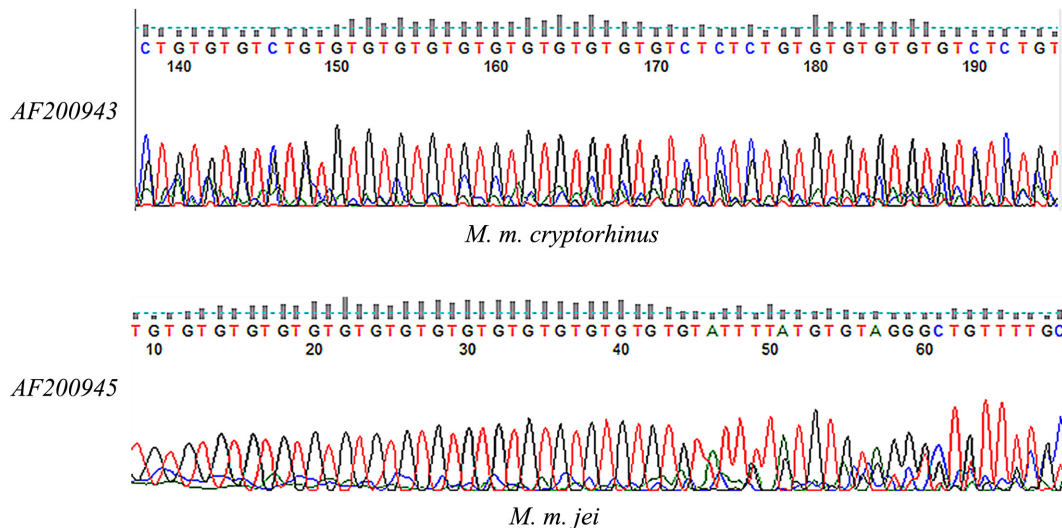

Fig. 4. Cloning sequences showing *Meriones meridianus* genomes generated at the loci *AF200943* (up) and *AF200945* (down) by Mongolian gerbils.

AF200945, and *AF200947* loci were significantly polymorphic. Based on the results of PCR, STR scanning, and sequencing, an appropriate microsatellite loci system was established.

Analysis of the genetic structure of *Meriones meridianus*

Samples of 39 *M. m. jei* and 24 *M. m. cryptorhinus* individuals were screened for the analysis of the ge-

Table 2. Summary of the statistics for 11 microsatellite loci in the two *Meriones meridianus* gerbil populations

Loci	M. m. jei							M. m. cryptorhinus							F_{ST}
	Obs-alle	Eff-alle	Shan-	Poly-loci%	Obs-Het	Exp-Het	Nei's	Obs-alle	Eff-alle	Shan-	Poly-loci%	Obs-Het	Exp-Het	Nei's	
AF200941	7	4.8907	1.7	–	0.8462	0.8059	0.7955	5	3.1911	1.2995	–	0.2083	0.7012	0.6866	0.0865
AF200942	7	2.5934	1.3315	–	0.6667	0.6224	0.6144	4	2.8444	1.1803	–	1	0.6622	0.6484	0.1355
AF200943	10	4.8517	1.871	–	0.8462	0.8042	0.7939	2	1.0425	0.1013	–	0.0417	0.0417	0.0408	0.3832
AF200944	12	5.5209	1.9866	–	0.7949	0.8295	0.8189	4	2.7961	1.1524	–	0.75	0.656	0.6424	0.0676
AF200945	7	4.8057	1.7002	–	0.6154	0.8022	0.7919	4	2.8872	1.1386	–	0.7083	0.6676	0.6536	0.1017
AF200947	8	6.6419	1.9467	–	0.8974	0.8605	0.8494	2	1.3318	0.4154	–	0.2917	0.2544	0.2491	0.2735
Mit22	3	2.7531	1.0559	–	0.9474	0.6453	0.6368	2	1.9692	0.6853	–	0.875	0.5027	0.4922	0.0606
D3Mit130	3	1.3011	0.4692	–	0.1026	0.2344	0.2314	2	1.0868	0.1732	–	0	0.0816	0.0799	0.7091
D8Mit56	1	1	0	–	0	0	0	2	2	0.6931	–	1	0.5106	0.5	0.6
D17Mit38	5	1.6751	0.8026	–	0.4615	0.4083	0.403	3	1.7916	0.6976	–	0.625	0.4512	0.4418	0.3641
Mit26	5	4.1674	1.4669	–	0.5789	0.7702	0.76	1	1	0	–	0	0	0	0.3114
Mean	6.1818	3.6546	1.3028	90.91%	0.6143	0.6166	0.6087	2.8182	1.9946	0.6852	90.91%	0.5	0.4118	0.4032	0.2585

Obs-alle, observed number of alleles; Eff-alle, effcetive number of alleles; Obs-Het, observed heterozygosisty; Exp-Het, expected heterozygosisty; Nei's, Nei's genetic variation; F_{ST}, fixation index, Shan-, shannon's information index analyzed for 11 loci.

netic structures of 11 polymorphic MS loci. Supplementary Table 1 shows the genotypes of each microsatellite locus in all of the samples. The average effective number of alleles, heterozygosity, and average observation of heterozygosity as well as other factors, were analyzed in the determination of genetic variation in each population. As shown in Table 2, not all loci were equally variable. The average observed numbers of alleles in the two populations were 6.1818 and 2.8182, the average effective numbers of alleles were 3.6546 and 1.9946, the average Shannon information indices were 1.3028 and 0.6852, the average observed heterozygosity values were 0.6143 and 0.5000, and the average expected heterozygosity values were 0.6166 and 0.4118, respectively. The average Nei's genetic distances were 0.6087 and 0.4032. The fixation index (F_{ST}) value, including all loci, was estimated to be 0.2585, which indicated a significant genetic differentiation between the *M. m. jei* and *M. m. cryptorhinus* populations.

Discussion

Meriones meridianus has been widely used as a valuable animal model in many medical fields [21, 31]. Its quality has an important influence on the accuracy, repeatability and scientificity of the research results. In the present study, we sought to construct a system for evaluating genetic quality using microsatellite markers for *Meriones meridianus* gerbil populations in China, which will be helpful for genetic quality control and monitoring of these populations. Molecular marker technology has brought with it the opportunity for the genetic breeding of experimental animals. For example, DNA sequencing [5], random amplified polymorphic DNA (RAPD) [17], amplified fragment length polymorphism (AFLP) [23], restriction fragment length polymorphisms (RFLP) [1],

and other technologies have become powerful tools for genetic diversity analysis. Among these molecular biomarkers, the stability of the RAPD technology has been inadequate, while RFLP and other methods have required large amounts of high purity DNA. In the present study, microsatellite DNA polymorphism markers were selected as primers on both sides of the site, and PCR amplification was performed with high repeatability and comparability. Therefore, microsatellite DNA polymorphism markers represent an ideal tool for genetic diversity studies. Screening of microsatellite markers from near-source species is a feasible method [25, 33]. This approach is particularly useful for species that do not have sequence information available. Mongolian gerbil is a gerbil strain that has been widely used in a variety of studies [4, 11, 28]. In a previous study, we have screened 28 microsatellite marker systems to determine the genetic quality of a closed group of Mongolian gerbils through the use of cross-amplifying technology, using microsatellite sites in mice [6]. The genetic detection system was effectively applied to population quality monitoring for Mongolian gerbils in that study. Since midday gerbils and Mongolian gerbils are the closely related species, after further experiments, microsatellite loci suitable for *Meriones meridianus* were screened from 28 loci of Mongolian gerbils in the present study.

The results showed that 11 pairs (39%) could be stably amplified and had a single amplified stripe in agarose gel electrophoresis. After gene analysis of synthetic fluorescent primers, 11 microsatellite loci with good polymorphism and high heterozygosity were obtained. The results showed that it is feasible to obtain microsatellite sites by cross-amplification between near-source species (*Meriones meridianus* and Mongolian gerbils).

In our research, genetic variation between the two *Meriones meridianus* strains was studied using 11 mic-

rosatellite markers. Genetic variation in the group was determined on the basis of average effective heterozygosity, average effective allele number, average Shannon index, and other analytical indices used for estimating group genetic variation.

Nei (1987) stated that the mean number of alleles for different populations is a fair indicator of genetic variation and mutation drift balance. A higher mean effective number of alleles indicate that a population is able to maintain the original gene and avoids new variation in the case of pressures from genetic drift and artificial selection [20, 29]. In the *M. m. jei* group, the observed number of alleles varied from 1 to 12, with a mean of 6.1818, whereas the effective number of alleles varied from 1 to 6.6419, with a mean of 3.6546. The results indicate that the alleles were stable and evenly distributed in the population. In the *M. m. cryptorhinus* group, the observed number of alleles varied from 1 to 5, with a mean of 2.8182, and the effective number of alleles was 1 to 3, with a mean of 1.9946. The effective number of alleles was less than the actual observed number of alleles, which indicates that the allele distribution of the population was uneven; however, the fact that the population of this group was so small, only 24 animals, may have affected the number of effective alleles of this group.

Average effective heterozygosity is an important index that reflects the genetic diversity of a population. When the average effective heterozygosity of a group is less than 0.5, its genetic diversity is low; low genetic variability (heterozygote deficiency) coupled with inbreeding is the consequence of genetic bottleneck resulting from overexploitation and habitat-related events. The heterozygosity of an ideal closed group is between 0.5 and 0.7. In the *M. m. jei* stock, the average observed heterozygosity (HO) was 0.6143, and the average expected heterozygosity (HE) was 0.6166. This suggests that the population had high genetic diversity, which may be due to long-term natural selection for adaptation, the mixed nature of the breeds, or historic mixing of strains of different populations [12]. In addition, it was found that the mean observed heterozygosity was similar to the mean expected heterozygosity, indicating that there was no naked allele or inbreeding within this population. The results of this research suggested that the hybrid standard was strictly followed during the breading process for this population, thus obtaining good genetic variation and diversity.

The above indicates that the *M. m. jei* group will be able to adapt to changing environments and could be the ideal closed group in the future. According to the existing data, *M. m. cryptorhinus* stock (mean heterozygosity=0.4118; mean allelic number=1.9946) with less genetic variability will be unlikely to resist selection, mutation, and genetic drifting. This is likely the result of rapid population expansion from a small population. In addition, not many microsatellite loci (11) and breeds (24) were analyzed within the *M. m. cryptorhinus* stock; therefore, additional markers and samples are required to increase the accuracy and generality of the results. Thus, breeding strictly with non-inbred strains, which can increase genetic variation, is necessary in the future.

The (F_{ST}) is the correlation between two gametes randomly drawn from each subgroup and measures the degree of genetic differentiation and the heterogeneity of gene frequencies in sites studied [19]. The value of F_{ST} can range from 0 to 1. Wright had stated that values ranging from 0–0.05 indicate "little" genetic differentiation, those from 0.05–0.15 indicate "moderate" genetic differentiation, and those from 0.15–0.25 indicate "great" great genetic differentiation [36]. The higher the F_{ST}, the greater the implied different of a population from other populations. In this study, F_{ST} values (F_{ST}=0.2585) indicated a significant F_{ST} genetic differentiation between the *M. m. jei* and *M. m. cryptorhinus* groups. According to genetic data, *M. m. jei* [34] belongs to *M. penicilliger* [10], and *M. m. cryptorhinus* from the south of the Taklamakan Desert belongs to *M. psammophilus* [10, 40]. Based on the distance and barriers, there is a low genetic flow between the two subpopulations, which can be attributed to the differences between the two groups.

Evaluation of genetic variation and patterns of population/species genetic diversity provides insight into the breeding methods, which could to rapid changes in methods and conservation. In this study, we developed a microsatellite loci system for identifying the stock of *Meriones meridianus*. However, the ideal microsatellite tag would ideally be able to meet certain conditions. For instance, Barker proposed the analysis of at least 25 samples for each variety. The number of microsatellites should not be less than 25 (4 alleles per locus), and there should be no association between the position points. Currently, 11 microsatellites are not sufficient to establish a perfect marker system; further studies on the candidate sites of *Meriones meridianus* are required to determine the genetic structure of their populations.

Use of highly polymorphic microsatellite markers as genetic tags plays an important role in monitoring *Meriones meridianus* as a laboratory animal model; for instance, in our research, microsatellite loci system were shown to be powerful markers for quantifying genetic variations within and between populations of *M. m. jei* and *M. m. cryptorhinus* species. Population gene fre-

quency is an important index of *Meriones meridianus* genetic variation sizes, which should be taken into account in the modeling period. It would be useful to keep the base genetic profiles of representative samples of *Meriones meridianus* in a holding facility.

In conclusion, there have been few genetic studies of microsatellite DNA markers in *Meriones meridianus*, since DNA markers are very rare. To date, no detailed information is available on the genetic diversity of cultured stocks of *Meriones meridianus*. In this study, we identified appropriate microsatellite markers for *Meriones meridianus* and elucidated ways to enrich their genetic database. Continuous monitoring of the genetic structure using microsatellite markers is essential for the successful propagation of *Meriones meridianus*.

Taken together, the combination of 11 loci selected in this experiment provides a good choice for genetic monitoring of the quality and the population genetic structure of outbred stocks.

Acknowledgments

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