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PRIMER NOTE

The highly polymorphic microsatellite markers for the greater long-tailed hamster (*Tscherskia triton*)

LAIXIANG XU, * MINGJING SONG,*‡ YU GUO, ‡ FANHUA KONG, ‡ and ZHIBIN ZHANG †
*College of Life Sciences, Qufu Normal University, Qufu 273165, Shandong, China, †State Key Laboratory of Integrated Management on Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100080, China, ‡Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences, Beijing, 100021, China

Abstract

We isolated 25 dinucleotide microsatellite loci from the greater long-tailed hamster (*Tscherskia triton*) populations in North China. We developed the amplification conditions of polymerase chain reaction for producing high-resolution genetic markers for each locus. We found 10 microsatellite loci were highly polymorphic in 90 individual hamsters from three areas of North China, and the number of alleles in each locus varied from three to 11. These markers are potential tools for studying the genetic variation of the natural populations of this species.

Keywords: genealogical changes, genetic structure, greater long-tailed hamster, microsatellite

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The greater long-tailed hamster (*Tscherskia triton*) is widely distributed in croplands of North China, southern Korea and southern Siberia, Russia (Luo *et al*. 2000). Little is known about the genetic population structure of the species. To examine the relationship between population dynamics and genetic structure of the species, it is essential to develop several effective genetic markers that provide sufficient statistical power to assess the temporal and spatial genetic variations and the genealogical changes among the individuals in its natural populations. In this study, we isolated 25 binucleotide microsatellites in *T. triton* and developed 10 microsatellite primer sets showing highly polymorphic and accurate bandings.

The great long-tailed hamsters were captured in the Guan, Shunyi and Raoyang Counties of the North China Plain. Genomic DNA was isolated from the liver tissue of *T. triton* and preserved in 95% ethanol using standard phenol–chloroform protocols (Sambrook *et al*. 1989). About 2.4 μg total genomic DNA of nine individuals from three populations of *T. triton* was partially restricted with *Sau*3AI (TaKaRa, Biotechnology). DNA fragments of 200–700 bp were retrieved and ligated to *Bam*HI-digested and dephosphorylated pUC19 vector (TaKaRa). Recombinant vector was transformed into *Eschericia coli* competent DH5α cells (TaKaRa). Recombinant clones were selected following polymerase chain reaction (PCR) technique-based screening method using one of vector specific primers and each of $(CA)_8$ $(CT)_8$ or $(AT)_8$ oligos matching the core repeats motifs of microsatellites as primer pairs (Isaksson & Tegelström 2002). PCR was carried out in 25 μL volumes (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 μM each primer, 1 U Ampli*Taq* DNA polymerase (TaKaRa), and about 50 ng DNA from bacterial suspensions) using a Biometra Tgradient 96 thermocycler (Sathe *et al*. 1991). After a 5-min, 94 °C denaturation step, 35 cycles were performed as follows: 1 min at 94 °C, 1 min at 42 °C, 1 min at 72 °C, and finally, a 10-min extension step at 72 °C. Seventy-four putative microsatellite positive clones screened out by the above method were prepared for sequencing. Sequencing was performed using ABI BigDye DNA Sequencing Kit (version 3.1) and the sequences were visualized on an ABI 3100 DNA sequencer. Twenty-five clones contained microsatellites. Primer pairs for 16 microsatellite loci screened out by the above method were designed using the program PRIMER 3, which developed by the Whitehead Institute for Biomedical Research (Rozen & Skaletsky 2000) and PRIMER PREMIER 5.0 (PREMIER Biosoft International). Ten of them

Laixiang Xu and Mingjing Song both contributed equally to the study.

Correspondence: Zhibin Zhang, Fax: (86) 10 62565689; E-mail: zhangzb@ioz.ac.cn

Table 1 Primer sequences and allele characteristics of *Tscherskia triton* microsatellites

Locus	GenBank Accession no.	Primer sequences (5′–3′; F, forward; R, reverse)	Repeat motif	T_a	Genotypes	A/size range (bp)	H_O/H_E
GYA66	AY780301	F: CCCAGGAATGTTTATC R: AAGCCACCTTACTGACCC	$(CT)_{25}(GT)_{21}$	53	60	6/435–474	0.68/0.51
GYA136	AY780304	F: CAGTCAGCCTTGTTCCAG R: CAAATGCCCTCTTAGTGT	$(CT)_4(CA)_4(TG)(CA)(TA)_2(CA)C(CA)_4(TA)(CA)T(TA)(CA)(CAA)_4$	54	90	6/148–185	0.27/0.35
GYA183	AY780297	F: GAACTGATGCCCTTGTGG R: CATTCCCTTATTGTCTGG	$(CA)_4$	51	90	5/339–368	0.38/0.41
GYA189	AY780309	F: AAACATAAATGGGAGACA R: CTAAACCTGAACTGAGC	$(AC)_{16}(AG)_{10}(A)_2(AG)_{10}$	55	60	5/253–275	0.66/0.62
GYB13	AY864068	F: ATGAAGGTAGAAAGAGGGAA R: TTATGAGTGGGGTGCTGA	$(CA)_{20}$	47	30	6/114–146	0.80/0.67
GYB47	AY864074	F: ATCCCTCTTCTCTCTTCTGG R: AAAGCACTACTACCTCTGA	$(CA)_{18}(GA)_{20}$	54	90	9/291–338	0.33/0.31
GYA185	AY780308	F: AAACAGGAACTATGGAGGCA R: TGGTATAATTTATTTGGTG	$(AC)_{14}$	55	90	11/329–354	0.55/0.64
GY103	AY780298	F: CTGGTCCTCTGAAAAG R: AACCTACTGCCTCTAT	$(GT)_{16}$	50	90	4/166–182	0.68/0.76
GYB28	AY864071	F: CCTCTGTCATCCCCAAGT R: AGAAACCCTGTCTCAAAA	$(CCTT)_{11}$	51	90	6/331–383	0.72/0.78
GYA181	AY780305	F: GGGCTGACTTACAGTTTTAG R: CAAGGTGGGCTTTGAGGT	$(GT)_{15}(AT)(GT)_{12}$	51	90	3/169–185	0.69/0.78

Note: Genotypes, refer to the number of actual genotypes obtained for each locus; A, H_E and H_O, refer to the allele number, expected and observed and heterozygosities, respectively; T_a, refers to annealing temperature (°C).

yielded high-quality polymorphic PCR products, while another primer pair failed to yield expected PCR products. The forward primers of the 10 primer pairs were 5′ end-labelled with HEX. A total of 90 individuals (30 individuals per population) of the three local populations were genotyped for each locus. PCR was carried out in 25 μL volumes (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5–2.5 mM $MgCl_2$, 0.2 mM each dNTP, 1 U Ampli*Taq* DNA polymerase (TaKaRa), 0.4 μM each primer, and 80 ng DNA template). DNA amplifications began with a 2-min denaturing at 94 °C, followed by 35 cycles of the respective thermal reaction containing: denaturing at 94 °C for 50 s, annealing at the appropriate temperature from 41 to 55 °C for 1 min, and extending at 72 °C for 1 min, with a final extension for 7 min at 72 °C. High-quality amplification products were determined using an ABI 377 sequencer in GENESCAN mode to detect the labelled primers in a 5% denaturing polyacrylamide gel and compared with a size-standard ladder (ABI GENESCAN LIZ500). Results showed that these 10 microsatellite primer pairs amplified reliable and polymorphic DNA bandings. PCR annealing temperature for the microsatellite loci were conducted under the conditions presented in Table 1.

Estimates of genetic polymorphism were obtained by assessing the frequency of each allele using GENEPOP version 3.1c (Raymond & Rousset 1995). Within each local population, neither linkage disequilibrium nor deviation from Hardy–Weinberg proportions ($P < 0.05$) could be detected using exact tests. The observed and expected heterozygosities of these loci ranged from 0.27 to 0.8 and from 0.31 to 0.78, respectively. The number of alleles at these loci ranged from three to 11 (Table 1).

The alleles in locus AY780301 and AY780309 were only detected in hamsters from the Guan and the Shunyi Counties, while the alleles in locus AY864068 were only found in hamsters from the Raoyang County. This observation implies that these markers are useful in distinguishing the individuals from different areas. Three primer sets for microsatellite loci (mau3, mau13 and mau14) in golden hamster (*Mesocricetus auratus*) and three primer sets for microsatellite loci (Ccrμ13, Ccrμ15 and Ccrμ17) in common hamster (*Cricetus cricetus*) proved to be highly polymorphic in the greater long-tailed hamster (Song *et al*. 2005).

In general, the microsatellite markers in the greater long-tailed hamsters of this study were found to be highly polymorphic, and are thus potential tools in studying genetic variations in populations of this species.

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References

Isaksson M, Tegelström H (2002) Characterization of polymorphic microsatellite markers in a captive population of the eagle owl (*Bubo bubo*) used for supportive breeding. *Molecular Ecology Notes*, **2**, 91–93.

Luo ZX, Chen W, Gao W (2000) *Fauna Sinica Mammalia in China*, pp. 63–75. Science Press, Beijing (in Chinese.)

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology*, **132**, 365–386.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sathe GM, O'Brien S, McLaughlin MM, Watson F, Livi GP (1991) Use of polymerase chain reaction for rapid detection of gene insertions in whole yeast cells. *Nucleic Acid Research*, **19**, 4775.

Song MJ, Zhang ZB, Neumann K, Gattermann R (2005) Sex-biased dispersal of greater long-tailed hamster (*Tscherskia triton*) revealed by microsatellites. *Canadian Journal of Zoology*, **83**, 773–779.