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TECHNICAL NOTE

Eighteen novel microsatellite markers for the greater long-tailed hamster (*Tscherskia triton*)

Chuanhai Li · Jingping Dong · Zhenlong Xiao · Zhibin Zhang

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Abstract Eighteen polymorphic microsatellite markers were developed for the greater long-tailed hamster (*Tscherskia triton*), a species of interest across northern China. The expected heterozygosity at these loci ranges from 0.6444 to 0.9196, with the observed allele numbers varying from 6 to 23. All loci conformed to Hardy–Weinberg equilibrium and no pair displayed linkage disequilibrium after a Bonferroni correction. These markers should prove useful for studies of population structure, kinship, social structure and other interesting issues of this important species.

Keywords Greater long-tailed hamster · *Tscherskia triton* · Microsatellite · Genetic markers

The greater long-tailed hamster (*Tscherskia triton*) is one of the major pest rodents throughout the plains of northern China. It is also widely distributed in the northern Korean Peninsula and eastern Russia (Luo et al. 2000). Recently, this species has been subjected to extensive ecological studies in pursuit of an integrated management strategy (Wang et al. 1996, 2002; Yang et al. 1996; Zhang et al. 1998, 2001); however, little is known about its population genetic background (but see Xie and Zhang 2005, 2006). Given the importance of *T. triton* to local agricultural ecosystems and the fact that it is a paradigm for understanding small mammal dynamics, comprehensive investigation of its population genetics involving microsatellite markers are now warranted.

Song et al. (2005) had found male biased dispersal in *T. triton* using six microsatellite markers obtained through cross-species amplification. More microsatellite loci will allow for in-depth population structure depictions, robust kinship inferences, and accurate mating system and social structure investigations (Blouin 2003; Ryman et al. 2006). For those purposes, we have developed 18 novel polymorphic microsatellite markers for *T. triton*.

Microsatellite loci were isolated using the enrichment method described by Hamilton et al. (1999). Total genomic DNA was extracted from the fresh liver tissue of a male greater long-tailed hamster collected from Gu'an County (Hebei, China) using a standardized phenol-chloroform protocol (Sambrook et al. 1989). The genomic DNA was then digested into 300–1000 bp fragments with *Rsa*I and *Xmn*I (New England Biolabs). The double-stranded Super-SNX linkers (SuperSNX24 Forward 5′-GTTTAAGGCCT AGCTAGCAGAATC-3′ and SuperSNX24 + 4P Reverse 5′-pGATTCTGCTAGCTAGGCCTTAAACAAA-3′) were ligated to digested DNA fragments, which were used to construct a linker-ligated DNA library. The microsatellite-containing fragments were screened and enriched by Dynabeads (Dynal Biotech) with 10 simple sequence repeat (SSR) probes: $(AG)_{12}$, $(AT)_{12}$, $(CG)_{12}$, $(GT)_{12}$, $(ACG)_{12}$, $(ACT)_{12}$, $(CCA)_8$, $(AACT)_8$, $(AAGT)_8$ and $(AGAT)_8$. To increase the amount of enriched fragments, a 'recovery' polymerase chain reaction (PCR) was conducted on the PTC-100 thermal cycler (Bio-Rad) using the following cycling profile: an initial 2 min denaturation step at 95°C,

C. Li · J. Dong · Z. Xiao · Z. Zhang (✉)
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Chaoyang, 100101 Beijing, China
e-mail: zhangzb@ioz.ac.cn

J. Dong · Z. Xiao
Graduate School of the Chinese Academy of Sciences, 100039 Beijing, China

Table 1 Characterization of 18 microsatellite loci in *Tscherskia triton*

Locus	Primer name and sequence (5′–3′)	Labeling dye	Repeat type	T_a	N_A	Size range (bp)	H_O	H_E
*Tsc*1	L8F: ACAGCTAAGATCAGTCAAATAC	F: 5′-TAMRA	$(CA)_6TACA(CTAT)_7$	54	11	276–300	0.6000	0.6444
	L8R: GAAATGCAGTAGTGAACAATAC							
*Tsc*2	L19F: TCATTGCAGTGCATAAACAG	F: 5′-TAMRA	$(GT)_8(GA)_{26}$	58	11	184–212	0.8049	0.8591
	L19R: CAACTCCCAAAAGTTGTCCT							
*Tsc*3	L20F: GGGCAACATAGTAATGTGTAAT	F: 5′-FAM	$(GA)_{23}$	54	11	184–204	0.6889	0.8517
	L20R: CCCTCCAACAGTCCTGAC							
*Tsc*4	L24F: TCCCTAGTTGTCCTAGATTA	F: 5′-TAMRA	$(TCTG)_5TCCATCCCCA(TC)_{13}(CA)_{14}$	54	14	235–267	0.8140	0.8722
	L24R: GTCTACTGAGCACAAACACTG							
*Tsc*5	L40F: TCAATTTCCGGAGACCCTACCT	F: 5′-FAM	$(AG)_{27}$	54	14	240–268	0.9111	0.9196
	L40R: GGGGACTAGACTAGGTCATGTG							
*Tsc*6	L65F: TGGCATTGACAGTTGGTTTAG	F: 5′-FAM	$(GA)_{20}$	54	6	190–200	0.6342	0.7612
	L65R: AAACAAAGAGCCTGGTTCACT							
*Tsc*7	L75F: CCACTGGCACAGAGTAGATTGT	F: 5′-FAM	$(AC)_7AT(AC)_4T(CA)_3T(AC)_{15}$	58	10	106–126	0.8409	0.8730
	L75R: ATTGACCCAGTTCCTTACG							
*Tsc*8	L78F: TTGAGGCCAACCTTAACTACAC	F: 5′-HEX	$(AC)_{24}TCACTC\ (AG)_{12}$	54	11	200–224	0.9111	0.8602
	L78R: AGGGGTCCTGGTGATTAAGTCT							
*Tsc*9	L92F: TCTCCCTTCCTTATTACCTTAC	F: 5′-HEX	$(AC)_{19}$	58	10	232–254	0.8000	0.8554
	L92R: GCCTGAACCTGTTTCTATG							
*Tsc*10	S31F: TGCCACCAACCTCTACAT	F: 5′-FAM	$(CA)_{12}C(GA)_{23}$	54	12	191–219	0.8667	0.8871
	S31R: CAAATCATAATATGGATGCC							
*Tsc*11	S33F: AGTTCACGAACATCACACTG	F: 5′-FAM	$(AG)_{29}$	54	11	257–279	0.8222	0.8812
	S33R: TTCCCAGTTGGTGGACTG							
*Tsc*12	S36F: AGTCTCACAGTATATTACCCTG	F: 5′-FAM	$(AG)_{15}(GT)_8$	52	9	237–253	0.6191	0.7932
	S36R: AGACAGATGTCCTGCTAACT							
*Tsc*13	S57F: TCTAGCCCCCTTTGTGT	F: 5′-FAM	$(TG)_{20}$	50	7	174–186	0.7805	0.8079
	S57R: CAATCTTGGGGAATTTATCT							
*Tsc*14	S72F: CATCGCCAATATCACAATC	F: 5′-FAM	$(AC)_7AT(AC)_4AA(AC)_5(AC)_{25}$	54	10	183–205	0.8222	0.8679
	S72R: GAAATCCCACCAGCTTATG							
*Tsc*15	S73F: GCTCATGATTGGTCGTTCC	F: 5′-FAM	$(GA)_{28}A(AG)_{10}$	50	14	236–280	0.7556	0.8769
	S73R: GGCAATTCTCTACTTAGGGTAT							
*Tsc*16	S81F: TAGATTCAGTTGTTGCATTGC	F: 5′-FAM	$(GA)_{28}A(AG)_{10}$	54	23	187–245	0.9333	0.9159
	S81R: TGCAAGTTGTCCTCTGACTACT							
*Tsc*17	S87F: CCAAGTGTCAACCTTTGGT	F: 5′-HEX	$(CA)_{23}GG(GA)_9$	54	15	199–231	0.9091	0.8997
	S87R: TGAGTCCACATTCATCTTG							

Table 1 continued

Locus	Primer name and sequence (5′–3′)	Labeling dye	Repeat type	T_a	N_A	Size range (bp)	H_O	H_E
*Tsc*18	S88F: ACAGCCCAGATTGTCCTCT	F: 5′-FAM	$(TC)_{29}$	54	11	143–165	0.8182	0.8495
	S88R: CACCATTCCTGGTCCAAC							

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The final annealing temperature (T_a) unit is °C. The number of alleles (N_A), allele size range in base pairs (bp), and observed (H_O) and expected heterozygosity (H_E) are listed for all loci

followed by 25 cycles of 95°C for 20 s, 60°C for 20 s and 72°C for 90 s, and a final extension at 72°C for 30 min. The reaction mixture (25 μl) included 1× PCR buffer (Tiangen Biotech), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of the SuperSNX24 forward primer, 1 U *Taq* DNA polymerase (Tiangen Biotech) and ~25 ng enriched genomic DNA fragments. The PCR products were then transformed into pGEM-T easy vectors (Promega). After incubation, positive (white) clones were selected and tested by PCR amplification following the cycling protocol above to determine whether target DNA fragments were inserted. Successful clones were sequenced using an ABI 3730 automated sequencer (Applied Biosystems).

A total of 180 microsatellite-containing sequences were detected from 186 positive clones. After clustering and assembly, 116 unique microsatellite sequences were found. Ninety-three primer pairs were designed using OLIGO 6.0 (Molecular Biology Insights). Polymorphisms of these new markers were tested using samples of 45 individuals captured from Harbin (Heilongjiang, China). PCR amplification was performed in a final volume of 20 μl containing 5 ng of genomic DNA, 1× PCR buffer (Tiangen Biotech), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer and 1 U *Taq* DNA polymerase (Tiangen Biotech). PCR was conducted on the PTC-100 thermal cycler (Bio-Rad) and the cycling profile was as follows: an initial denaturation step of 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 30 s at a primer-specific annealing temperature (T_a°C, Table 1) and 45 s at 72°C, and finished by a final extension at 72°C for 5 min. Forward primers were labeled with FAM, HEX or TAMRA in the 5′ end (Table 1). All PCR products were checked using an ABI 377 automated sequencer with GeneScan ROX 500 size standard and analyzed with GeneScan 3.7 (Applied Biosystems).

The number of alleles (N_A), expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated using Arlequin 3.1 (Excoffier et al. 2005). The data set was checked for errors and null alleles using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Tests for linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP 3.4 (Raymond and Rousset 1995).

Eighteen microsatellite loci were found to be polymorphic and the number of alleles varied from 6 to 23. Table 1 lists sequences of these primers, the key experimental parameters for PCR amplification and some population genetic estimates. Observed and expected heterozygosity varied from 0.6000 to 0.9333 and from 0.6444 to 0.9196, respectively. Null alleles were only detected at loci *Tsc*8 (99% confidence). No locus was found to deviate from HWE ($P < 0.01$) and no loci pair displayed linkage disequilibrium ($P < 0.01$) after a Bonferroni correction (Rice

1989). Microsatellite loci developed here and before (Song et al. 2005; Xu et al. 2007), will be useful for studies of genetic diversity, population structure and other interesting issues of *T. triton*.

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