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PERMANENT GENETIC RESOURCES

Eight polymorphic microsatellite markers developed in the Chinese scorpion, *Mesobuthus martensii* (Scorpiones: Buthidae)

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Abstract

Eight polymorphic di- and trinucleotide microsatellite loci were developed in the Chinese scorpion, *Mesobuthus martensii*. The expected heterozygosity at these loci ranges from 0.019 to 0.860, with the observed allele numbers varying from two to 25. Overall, there were no deviations from Hardy–Weinberg equilibrium and no observed linkage disequilibrium after Bonferroni correction. Cross-species amplification of these loci in *Mesobuthus eupeus* revealed that five loci can amplify successfully in this species. The polymorphic microsatellite DNA markers reported here should provide helpful means to address questions concerning phylogeographical patterns and evolutionary history of *M. martensii* and closely related species.

Keywords: *Mesobuthus eupeus*, *Mesobuthus martensii*, microsatellite, population genetic structure, scorpion

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Scorpions are among the few 'living fossils' known to be highly conservative in morphology (Sissom 1990) and widespread in distribution accommodating almost all kinds of habitats (Fet *et al*. 2000), with the fossils of some species dated back to the Silurian Period (ranging from 438 to 410 million years ago) (Kjellesvig-Waering 1966; Polis 1990). Moreover, these creatures have weak dispersal ability (Polis *et al*. 1985) and are important players in ecological trophic cascade. With such fascinating characteristics, scorpions qualify as excellent model organisms for phylogeographical and evolutionary study. Up to date, 29 species and subspecies have been reported in China (Zhu *et al*. 2004; Shi & Zhang 2005; Shi *et al*. 2007; and update references therein), with four of them belonging to genus *Mesobuthus* Vachon 1950. *Mesobuthus martensii* (Karsch, 1879), commonly called the 'Chinese scorpion', is the most widespread and most toxicologically studied species in China. Also, it has been popularly used in traditional Chinese medicine for more than 1000 years (Shi *et al*. 2007).

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Here, we report eight polymorphic microsatellite DNA loci isolated from this scorpion.

The enrichment method used for isolation of the microsatellite loci has been described in Ji *et al*. (2003). In total, 1152 recombinant clones were screened with biotin-labelled oligonucleotide probes using CDP-Star Universal Detection Kit (Sigma), and 181 positive clones were found. Inserts of positive clones were isolated using polymerase chain reaction (PCR) amplification directly from bacterial colonies using M13 universal forward and reverse sequencing primers (–47 and –48, respectively, New England Biolabs), then sequenced with ABI BigDye Terminator Cycle Sequencing Kit (version 2.0) in an ABI PRISM 3100 automated sequencer. Sequencing analysis showed that 12.4% of the recombinant clones contained microsatellite DNA.

Twenty positive clones were finally selected to design oligonucleotide primers, using OLIGO 6.31 primer analysis software (National Biosciences Inc.). Oligos were synthesized by Sangon Biotech. After extensive optimization, we found that 10 pairs of primers gave satisfactory results and each appeared to be a single-copy sequence in the genome. Further analysis with high-resolution Metaphor agarose gel (FMC Marine) electrophoresis suggested that these 10

pairs are likely to be polymorphic in the scorpion population. Nine of them were further tested by genotyping more than 70 Chinese scorpion individuals on an ABI PRISM 3100 automated sequencer using Pop4 gel matrix with GeneScan 400HD (ROX) as the internal size standard. The other one locus was tested by genotyping 45 Chinese scorpion individuals. Finally, eight loci are found to be polymorphic. The optimal PCR conditions employed for these loci on PerkinElmer GeneAmp 9700 thermocycler are as follows: a 10-μL reaction containing 20–30 ng of template DNA, 0.2 mM of each dNTP, 1× PCR buffer (Dingguo Biotech), 1.2–2.4 mM Mg^{2+}, 0.3 or 0.6 U of *Taq* DNA polymerase (Dingguo Biotech), and 0.3 μM of each primer were denatured at 94 °C for 4 min, then followed by 40 cycles of 20 s at 95 °C, 30 s at the appropriate annealing temperature (Table 1), and 10 s at 72 °C. The reaction was terminated by a final extension of 2 min at 72 °C.

Table 1 shows the characteristics of these eight microsatellite loci. The observed allele numbers vary from two to 25. The expected heterozygosity at these loci (calculated using the program GENEPOP, Raymond & Rousset 1995) ranges from 0.019 to 0.860 while the observed heterozygosity from 0.019 to 0.492. The apparent deficiency of heterozygotes was owing to that the genotyped samples were pooled from several populations covering a large geographical area that certainly includes more than one panmictic unit. When individual populations were used as the analysis unit, the locus MmSSR6 was found to deviate from Hardy–Weinberg equilibrium ($P < 0.05$; using ARLEQUIN version 3.0, Excoffier *et al*. 2005) in some populations after Bonferroni correction (Rice 1989). As expected, the presence of null alleles was also detected at locus MmSSR6 in these populations ($P > 0.05$; tested using the software MICRO-CHECKER, van Oosterhout *et al*. 2004). No linkage disequilibrium (ARLEQUIN version 3.0) was detected ($\alpha = 0.01$) among all possible pairwise locus comparisons after Bonferroni correction.

Cross-species applicability of the microsatellite loci developed here was tested in a geographically neighbouring congeneric species *Mesobuthus eupeus* (C. L. Koch, 1839) (Shi *et al*. 2007). Five of the eight loci can amplify successfully in this species, including MmSSR2, MmSSR4, MmSSR5, MmSSR6 and MmSSR8 (Table 1). However, MmSSR2 can only amplify a single weak band in *M. eupeus*.

A range-wide survey of the Chinese scorpion in our laboratory has revealed an unexpectedly high mitochondrial (mt) DNA haplotype diversity (over 200 mitotypes were observed, C. M. Shi & D. X. Zhang, unpublished data). Yet, little genetic difference and phylogeographical pattern can be detected at local scale, which should indicate the inappropriateness of the mtDNA marker and the significance of relatively recent ecological and evolutionary forces. The highly polymorphic microsatellite DNA markers reported here should provide useful means to address such questions

Table 1 Characteristics of eight polymorphic microsatellite loci in *Mesobuthus martensii*

										Cross-species amplification in *M. eupeus*	
Locus	Primer name	Primer sequence (5′–3′)	Repeat type	Labelling dye	T_a (°C)	$MgCl_2$ (mM)	No. of alleles	Size range (bp)	H_E	H_O	Specific product	T_a (°C)
MmSSR1	AA01F*	GTTTCTACCCTCTCGCTGTTG	Interrupt CAT	HEX	53	1.8	3	257–263	0.030	0.023	–	na
	AA01B	TTTAGTGTTCCTAGATGGTTATG										
MmSSR2	AB03F*	AGTGGTAAGGAGATAATCTGATAA	$(ATAC)_7AT$	HEX	55	1.2	25	111–167	0.860	0.492	w	56
	AB03B	TTTGCTTTGCATATGGTTCTTGT										
MmSSR3	BE10F*	CCACAACAGCATACGCACAA	$GTATGCAT(GTAT)_6$	FAM	51	1.8	2	179–188	0.066	0.036	+	56
	BE10B	GAACCTATGGCATGCAGAACAA										
MmSSR4	DE03F	GTGTTGTGATTTTGCTGTAAGT	$(GT)_6GC(GT)_2$	FAM	55	1.5	2	178–180	0.019	0.019	+	60
	DE03B*	AAATCAGAATGGATGTAGGTAG										
MmSSR5	DF10F*	TTCTATATCGTCGAAATGGTAA	$(TA)_2(TG)_{11}$	NED	50	2.4	22	109–146	0.848	0.451	+	56
	DF10B	TAGCTGGATAATCGAAATATCTG										
MmSSR6	EE10F*	TTTCCCTCCATTGTAATGAAC	$(ATTG)_6$	HEX	54	2.0	8	112–137	0.720	0.380	–	na
	EE10B	ATCGCAACGCCTGAAATGAT										
MmSSR7	EE12F	GTTGCAGTATTCCAATCACTC	$(CR)_8TA(CD)_6$	NED	53	2.0	7	120–132	0.411	0.192	–	na
	EE12B*	TAATCATTTTCAGCCTTGTTCTA										
MmSSR8	HF10F	TATTTTCATCTGCCGTTCAATC	$(TC)_{10}TT(TC)_2(TA)_2$	FAM	50	2.0	11	80–96	0.735	0.442	+	56
	HF10B*	AAGAGCACTTTAGGAAACACTT										

Primer sequences, repeat unit structure, expected (H_E) and observed (H_O) heterozygosities, number of alleles, optimal PCR annealing temperature (T_a) for PerkinElmer GeneAmp 9700 and the $MgCl_2$ concentration are indicated for each locus. *Denotes primers that are labelled with fluorescent dyes, '+' denotes one strong specific band being amplified in *Mesobuthus eupeus*, 'w' a single weak band and '–' no product, 'na' not applicable. EMBL accession nos AM889498–AM889505.

in *M. martensii*. In addition, mtDNA study has not detected any introgression between *M. martensii* and *M. eupeus* in the contact zone recently discovered by Shi *et al*. (2007). The availability of microsatellite markers will allow us to further investigate this interesting issue from the nuclear context.

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