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PRIMER NOTE

Ten polymorphic microsatellite DNA loci for paternity and population genetics analysis in the fen raft spider (*Dolomedes plantarius*)

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Abstract

Ten polymorphic di- and trinucleotide microsatellite loci were developed in the fen raft spider *Dolomedes plantarius* from a partial phagemid genomic library enriched for microsatellite inserts. The expected heterozygosity at these loci ranges from 0.62 to 0.9, with the observed allele numbers varying from four to 15 in the 22 individuals tested. Average paternity exclusion probabilities ranged between 0.290 and 0.686. In combination, the 10 polymorphic loci elicit an exclusion probability of 0.999. The high level of polymorphism of these microsatellite loci makes them ideal genetic markers for paternity and population genetics analysis in this endangered species.

Keywords: *Dolomedes plantarius*, fen raft spider, microsatellite, paternity, population genetics

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The fen raft spider, *Dolomedes plantarius*, is a semiaquatic endangered species, occurring at only three sites in Britain: the Pevensey Levels in Sussex, near Swansea in South Wales and Redgrave and Lopham Fen National Nature Reserve in Norfolk. At the latter site, its population is close to extinction. The species appears to be declining and endangered over much of western, central and eastern Europe (reviewed in Smith 2000). Various aspects of its life history suggest that water availability and water quality are the over-riding factors in the decline of the species (Smith 2000). At Redgrave and Lopham Fen, it is suspected that the raft spider has gone through several tight population bottlenecks caused by artesian abstraction exacerbated by repeated droughts. To set up an effective strategy for its management and conservation, we need to understand several questions at the population genetic level. For example, what genetic impacts do the bottlenecks have on the raft spider populations, how different genetically are the three *D. plantarius* populations in Britain and what is the reproductive behaviour of the raft spider and its connection to population recovery? Clearly, highly polymorphic genetic markers should be used to address these questions. Microsatellite DNA provides the most revealing nuclear markers available so far for population genetic analysis (Zhang & Hewitt 2003) and is thus the marker system of choice for the fen raft spider. Here we report the isolation and characterization of 10 polymorphic di- and trinucleotide microsatellite DNA loci in *D. plantarius*.

A partial genomic library with short inserts (300–800 bp on average) enriched for microsatellite DNA was constructed using an established method (Ji *et al*. 2003; Zhang *et al*. 2003) modified from Karagyozov *et al*. (1993). In total, 1152 recombinant clones were screened with biotin-labelled oligonucleotide probes using a CDP-Star™ Universal Detection Kit (Sigma) and 203 positive clones found with strong or quite strong hybridization signals. Inserts of positive clones were isolated using polymerase chain reaction (PCR) amplification directly from bacterial colonies with M13 universal and reverse sequencing primers (–47 and –48, respectively; New England Biolabs) and then sequenced with ABI BigDye™ Terminators Cycle Sequencing Kit (version 2.0) in ABI PRISM3700 or PRISM3100 automated sequencers. Sequencing analysis conclusively revealed that 85 of 102 positive clones contain real microsatellite sequences. This gives an overall microsatellite-cloning efficiency of 15% in *D. plantarius*.

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Table 1 Characteristics of 10 polymorphic and four monomorphic microsatellite loci in *Dolomedes plantarius*

Locus	Primer name	Primer sequence (5′–3′)	Repeat type	T_a (°C)	Labelling dye	No. of alleles	$MgCl_2$ (mM)	Size range (bp)	H_E	H_O
DpDC4	DC4F*	GTCAGCCTACTTTGTATATGT	$(YT)_{33}$	53	FAM	15	2	100–146	0.896	0.546
	DC4B	TGCAGGCATTTCCCGTTATT								
DpGC1	GC1F*	GAATTAACGAGCGTCTACTG	$(CA)_{11}$	52	FAM	5	2	137–149	0.705	0.682
	GC1B	CTAACAACCGAAATATATAC								
DpHF5	HF5F*	GAAGCAGCTCTCAATTCATTT	$(CT)_{12}GT$	53	HEX	12	2	165–211	0.870	0.636
	HF5B	AGAGGTTGCGTTGAGGACTTT	$TT(GT)_8$							
DpLD6	LD6F*	GGTTGCAAAATAAATGAGACAC	$(AT)_4(GT)_7$	52	FAM	4	2.5	220–226	0.617	0.524
	LD6B	TATCAGAATGTTCGCAGAGTA								
DpLE11	LE11F*	GCTCGCTTTCTCTCAACTAC	$(TG)_2(TA)_4(TG)_{10}$	53	HEX	6	1.5	287–307	0.785	0.714
	LE11B	CTTATGCCGCTTATTTGAAC								
DpMF6	MF6F*	TAATGCCACGATATAAATTATCC	$(CA)_9CG(CA)_3TAC$	52	FAM	10	2	178–204	0.849	0.682
	MF6B	TTATAACGAGCCACAAGAGAG	$A(TA)_6[(CC)(CA)_3]_2$							
DpNA1	NA1F*	TCCAATAGTTACGCTAAACA	$(CA)_{12}$	53	HEX	10	2.5	78–96	0.733	0.571
	NA1B	CAACCCTTCAATTTAGTTAT								
DpNH1	NH1F	TCTAGCGAATCCTTAAATATCC	$(CR)_{12}CT$	53	FAM	6	2	142–172	0.764	0.790
	NH1B*	AGTCTAGCTCCAGCCATGTAAA	$(CA)_2(TA)_3$							
DpRB5	RB5F	TTAATTTTCAATGTAGGGTATATC	$(CT)_{19}TT(CT)_2$	55	HEX	8	2.5	134–154	0.801	0.500
	RB5B*	GAAAGAATAATACATGCACTTAAC								
DpRE6	RE6F*	TTCCCAAACCAGCAGCAAGAG	$(TC)_{21}(TG)_2(TC)_4$	53	FAM	11	2.5	158–220	0.867	0.364
	RE6B	TGCCTGTGTCAAGTTCGTATC								
DpHH8	HH8F	ATGGAGGCTATGTGGATGTT	$(TG)_8$	53	HEX	1	1.5	185	ML	ML
	HH8B*	GATGGAATTGTGATGTCTACT								
DpKA6	KA6F	TGACTCAGCCACATTTGAACA	$(GAR)_7$	51	HEX	1	1.5	238	ML	ML
	KA6B*	GGATAATTTAAGGGAACAATAC								
DpLF12	LF12F	CGACGGAAGGAATGATTTAC	$(GT)_2(AT)_3$	53	HEX	1	1.5	205	ML	ML
	LF12B*	GCATGCAATTTTTACTCATTATAC	$(GT)_5GC(GT)_4$							
DpMG6	MG6F*	TTTTGATGAGGTCCGCTTCTA	$(TG)_4CG(TG)_7$	55	FAM	1	1.5	267	ML	ML
	MG6B	ATCCGCAAGCAAAGAATTAAC								

Primer sequences, repeat unit structure, expected (H_E) and observed (H_O) heterozygosities, no. of alleles, polymerase chain reaction annealing temperature (T_a) for GeneAmp 9700 (Perkin-Elmer) and the $MgCl_2$ concentration are indicated for each locus.
*Primers that are labelled with fluorescent dyes. ML, Monomorphic locus. GenBank Accession nos: AJ616858–AJ616871.

Twenty-four clones with a microsatellite longer than nine repeats in the inserts were selected to design oligonucleotide primers using OLIGO®6.31 primer analysis software (National Biosciences Inc.). Oligonucleotides were synthesized by MWG Biotech-UK or Genecore Biotech (Shanghai). After extensive optimization, 10 sets of primers were found to amplify microsatellite loci that are present in multicopy in the genome and are thus not suitable for use as molecular markers in population genetic studies. With the remaining 15 pairs, one primer of each pair was end-labelled with a fluorescent dye, either 6-FAM or HEX (Table 1), and further tested by genotyping with 22 raft spider individuals on an ABI PRISM3100 machine with GENESCAN-400HD (ROX) as the internal size standard. The PCR conditions employed are as follows: a 10-μL reaction containing about 30 ng of template DNA, 0.2 mM of each dNTP, 1× PCR buffer (HuaMei Biotech), 1.5–2.5 mM Mg^{2+} (Table 1), 0.3 U of *Taq* DNA polymerase (HuaMei Biotech) and 0.3 μM of each primer was denatured at 94 °C for 4 min, followed by 30–35 cycles of 20 s at 94 °C, 30 s at the appropriate annealing temperature (Table 1) and 15 s at 72 °C. The reaction was terminated by a final extension of 2 min at 72 °C.

Fourteen loci were found to have typical single copy nuclear locus characteristics, with 10 of them being polymorphic in the 22 individuals genotyped. The remaining four loci were monomorphic, although they may show polymorphism when more samples are tested. Table 1 summarizes characteristics of the 14 microsatellite loci of *D. plantarius* based on genotyping data from 22 individuals from the Redgrave and Lopham Fen and Pevensey Levels populations. The expected and observed heterozygosities were calculated using the program GENEPOP (Raymond & Rousset 1995). The expected heterozygosity at these loci ranges from 0.62 to 0.9 with the observed allele numbers varying from four to 15 in the limited number of individuals tested. This preliminary result suggests that, although endangered, each of the two British raft spider populations still maintains quite high genetic and allelic diversities. The average paternity exclusion probability for single loci

ranges between 0.290 and 0.686. One locus (DpRE6) deviates significantly from Hardy–Weinberg equilibrium possibly due to the presence of a null allele. In combination, the 10 polymorphic loci elicit an exclusion probability of 0.999, estimated with the program CERVUS 2.0 (Marshall *et al*. 1998). Therefore, these microsatellite loci are highly polymorphic and will provide an ideal genetic marker system for population genetics and paternity analysis in this endangered species.

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