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PRIMER NOTE

Ten polymorphic microsatellite markers developed in the masson pine moth *Dendrolimus punctatus* Walker (Lepidoptera: Lasiocampidae)

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Abstract

A set of 10 polymorphic di- and trinucleotide microsatellite loci were developed for the forestry pest insect, masson pine moth, *Dendrolimus punctatus* Walker. The expected heterozygosity at these loci ranges from 0.285 to 0.859, and the observed allele numbers from five to 19. Cross species amplification of these loci in four other congeneric pine moth species indicates variable levels of loci conservation and thus cross-applicability. Therefore, the microsatellite loci reported here should be useful for population genetic and other related studies in the masson pine moth and other closely related species.

Keywords: *Dendrolimus punctatus*, Lepidoptera, microsatellite DNA family, pine moth

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The masson pine moth *Dendrolimus punctatus* is one of the major forestry pest insects in Central and South China, causing great economical damage. The main host of *D. punctatus* is the masson pine (*Pinus massoniana*), but occasionally it also feeds on other pines such as the Chinese hard pine (*Pinus tabulaeformis*) and the swamp pine (*Pinus elliottii*) (Zhang & Zhao 1996). The distribution of the masson pine moth is restricted to latitude south of 33 °N in China. Sequential outbreaks occurred in geographically non-overlapped areas have been observed for this species. Therefore, it has been suspected that adults of this insect may be able to carry out long-distance dispersal. But there lacks evidence for this mainly because traditional methods (such as the 'capture, mark and release' method) to study dispersal and migration appeared to be inefficient. Thus a population genetic survey using highly polymorphic DNA markers may be a more effective approach, and microsatellite DNA should be the marker system of choice for such study. Additionally, some 25 congeneric pine moth species have been defined in East and South-East Asia based almost exclusively on morphological characters, with most of them being endemic to China (Hou 1987). Many of these congeneric species are also serious forestry pest insects. Thus, microsatellite DNA loci developed for the masson pine moth are likely to be useful for other pine moth species in both population genetics and systematics studies. Here we report 10 polymorphic diallelic microsatellite DNA loci isolated from the masson pine moth. Cross-amplifiability of these loci has also been tested in four closely related species.

The 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 oligo nucleotide probes using CDP-Star™ Universal Detection Kit (Sigma), and 121 positive clones were found. Inserts of positive clones were isolated using polymerase chain reaction (PCR) amplification directly from bacterial colonies using M13 universal and reverse sequencing primers (–47 and –48, respectively, New England Biolabs), then sequenced with ABI BigDye™ Terminators Cycle Sequencing Kit (version 2.0) in ABI PRISM 3100 automated sequencers. Sequencing analysis finally revealed an overall microsatellite-cloning efficiency of 10.5%, which is much higher than that in the cotton bollworm (2.5–3.8%) isolated with a similar method (Ji *et al*. 2003; Ji & Zhang 2004; Ji *et al*. 2005). Two microsatellite DNA families were identified, similar to that in the cotton bollworm.

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Table 1 Characteristics of 10 polymorphic microsatellite loci in *Dendrolimus punctatus*. 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. EMBL nucleotide sequence database Accession nos: AJ972593–AJ972602

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
DpSSR1	AE05F*	ACTTCTACTGCGTGTGAACT	$(AC)_{15}$	FAM	51	1.5	19	169–217	0.859	0.622
	AE05B	GTCCCTTTGTCCGATAATATG								
DpSSR2	AF04F*	GGAGCACCAATGAAGAATGT	$(CAT)_5CGT(CAT)_2CAC$	HEX	52	1.75	12	209–284	0.376	0.337
	AF04B	GTTTCTACCTCATGGGATCTTT								
DpSSR3	BC03F	CTGGCACCCTCTATTTATCT	$(CA)_9$	FAM	50	2.0	6	123–137	0.535	0.371
	BC03B*	AACAAAACAATTATAAACTCTTAC								
DpSSR4	BF09F*	TCATCCCGAGTCCCACTCA	$(CA)_{13}$	HEX	52	1.75	7	88–100	0.768	0.406
	BF09B	ATTGCTCTTCCTATCTGGCTA								
DpSSR5	CA04F	TTTGTCAGTTGGGATGATATT	$(GT)_3TT(GW)_5AA(SY)_9$	HEX	52	1.5	14	182–244	0.839	0.668
	CA04B*	ACACTGATACTCGGTCACATT	$AA(RT)_4T(GT)_6ATG(GY)_6$							
DpSSR6	DB02F*	TAGCTCACGTAAAACTAATCAA	$(CA)_{10}$	FAM	52	1.5	9	178–212	0.797	0.622
	DB02B	CTGTCCAAAGCAAACTATC								
DpSSR7	DH12F	CTGCTAGAGCTTTCTGTGTT	$(AT)_2GG(GY)_5ATG$	NED	50	1.5	15	132–178	0.797	0.417
	DH12B*	AAGAATTTCAATTTAAGACTGAC	$T(GC)_5CT(GT)_{12}$							
DpSSR8	GH09F	ACCAACTTCGACACCTTCT	$(GAT)_8$	NED	50	1.75	11	215–275	0.814	0.198
	GH09B*	CACTGCCCCGAACCTATAC								
DpSSR9	LG02F	ATCACACTCGCATTATTATAC	$(GT)_7GC(GT)_3(GC)_3(GT)_3GC$	HEX	46	1.75	9	185–215	0.744	0.220
	LG02B*	GATGAACGCCTATTAACATAC								
DpSSR10	MD04F	GTTCTCGGTCGTGGTTTTAG	$(GTN)_{19}GCT(GTA)_2$	NED	50	1.75	5	132–156	0.285	0.296
	MD04B*	AACCGCTTCCGCCGATTAC								

Twenty-three 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 11 pairs amplify microsatellite loci that are present in multicopy in the genome and thus dropped from further analysis. With the remaining 12 pairs, one primer of each pair was end-labelled with a fluorescent dye, either 6-FAM, HEX or NED (Table 1). They were further tested by genotyping with 96 masson pine moth individuals on an ABI PRISM 3100 automated sequencer with GeneScan-400HD (ROX) as the internal size standard. Ten loci show typical characteristics of polymorphic single copy nuclear loci, the other two loci appear to be duplicated or multiplicated in the genome. The final PCR conditions employed for these loci are as follows: A 10 µL reaction containing 20–30 ng of template DNA, 0.2 mM of each dNTPs, 1 × PCR buffer (HuaMei Biotech), 1.5–2.0 mM Mg^{2+}, 0.6 U of *Taq* DNA polymerase (HuaMei Biotech), and 0.3 µM of each primer, was denatured at 94 °C for 4 min, then followed by 30–38 cycles of 20 s at 94 °C, 30 s at the appropriate annealing temperature (Table 1), and 10–15 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 10 microsatellite loci. The expected heterozygosity at these loci (calculated using the program GENEPOP, Raymond & Rousset 1995) ranges from 0.285 to 0.859. The observed allele numbers vary from five to 19. The across-loci's consistently smaller observed heterozygosity shown in Table 1 should be mostly due to population subdivision, because the samples genotyped were randomly selected from different populations, and preliminary analysis of genotyping data already suggests that the masson pine moth populations in South China are significantly different from that in Central China. Therefore, these microsatellite loci can be employed as useful polymorphic markers in population genetic studies.

Cross species applicability of the microsatellite loci developed here was tested in four other pine moth species (Table 2). It can be seen that eight of 10 loci can amplify successfully in at least one other species, with three loci (DpSSR4, DpSSR8, DpSSR10) being successful in all species tested.

Previously, it has been recognized that the isolation of microsatellite loci is rather difficult in many lepidopteran insects compared to other insects (Nève & Meglécz 2000; Ji *et al*. 2003; Zhang 2004). For example, fewer useful loci can be isolated; microsatellite DNA family is more abundant; and there are a lower percentage of single-copy microsatellite loci (Ji & Zhang 2004; Zhang 2004). But exceptions also exist, such as the silk worm (Reddy *et al*. 1999). Our data suggest that the masson pine moth may be another exception in the cloning efficiency and the percentage of single-copy polymorphic loci finally optimized. Nevertheless,

Table 2 Cross amplification of the 10 *Dendrolimus punctatus* microsatellite loci in four other pine moth species. Meaning of symbols: '+' denotes one strong specific band being amplified, 'w' a single weak band, 'm' several weak bands and '—' no product

Locus	*D. punctatus*	*D. tabulaeformis*	*D. spectabilis*	*D. superans*	*D. kikuchii*
DpSSR1	+	+	—	+	+
DpSSR2	+	+	w	m	—
DpSSR3	+	+	+	—	+
DpSSR4	+	+	+	+	+
DpSSR5	+	m	—	—	—
DpSSR6	+	+	—	+	—
DpSSR7	+	+	w	+	—
DpSSR8	+	+	+	+	—
DpSSR9	+	m	—	—	—
DpSSR10	+	+	+	+	+

the proportion of microsatellite loci that is present in multiple copies in the genome is still high in the masson pine moth compared to other species (Ji & Zhang 2004).

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