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PRIMER NOTE

Isolation, characterization and cross-species amplification of eight microsatellite DNA loci in the migratory locust (*Locusta migratoria*)

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Abstract

Eight polymorphic di- and trinucleotide microsatellite loci suitable for population genetic analysis were developed in *Locusta migratoria* from a partial phagemid genomic library enriched for microsatellite inserts. The expected heterozygosity at these loci ranges from 0.45 to 0.97, with the observed allele numbers varying between nine and 45. The overall microsatellite cloning efficiency in *L. migratoria* is 14%, suggesting that in migratory locusts, microsatellite sequences are abundant and should provide a valuable and easily accessible source of nuclear markers for genetic studies. These microsatellite loci were highly *Locusta*-specific, with only very limited cross-species applicability.

Keywords: grasshopper, *Locusta migratoria manilensis*, migration, Orthoptera, repetitive microsatellite locus

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The migratory locust, *Locusta migratoria* L. (Orthoptera: Acridoidae) is one of the most important agricultural pests in the world. Occurring throughout Asia, Europe, Africa and Australasia, it is well known for its swarming behaviour, the ability to carry out long-distance migration, and continuous threats to agriculture (Centre for Overseas Pest Research 1982). Great efforts have been made in the study of this species during the whole of the twentieth century in order to find effective strategies and methods for its control. Whilst this has led to great understanding of its basic biology and ecology (see Uvarov 1966, 1977; Guo *et al*. 1991), it is clear that more research is needed to understand in sufficient detail many important aspects of this organism, such as their migratory behaviour, mating behaviour, swarming behaviour, temporal and spatial population dynamics and evolutionary trends. The employment of molecular genetic markers would seem particularly valuable for this.

One distinct characteristic of this species is its great migratory ability, presumably leading to frequent gene flows between populations. Therefore any genetic markers employed must be highly variable, otherwise they will fail to detect any individual- or population-level genetic variation. For example, we have already tested some nuclear noncoding regions (including the ribosomal internal transcribed spacer regions and some introns) and found they do not contain enough genetic variation for this task (unpublished data). In addition, previous studies have shown that mitochondrial pseudogenes exist in high copy number in the nuclear genome of *L. migratoria* (Gellisen *et al*. 1983; for review see Zhang & Hewitt 1996), thus making the use of mitochondrial DNA markers problematic in this species. Therefore, microsatellite DNA markers seem to be the sole practically accessible candidates. We report here the cloning and characterization of a set of eight diallelic microsatellite DNA markers from *L. migratoria*.

A partial genomic library with short inserts (300–800 bp, on average) enriched for microsatellite DNA was constructed using a method modified from Karagyozov *et al*. (1993). The same enrichment procedure was previously used for developing microsatellite loci in two gadoid fishes and the cotton bollworm (Zhang *et al*. 2001; Ji *et al*. 2003). It involves the selection of microsatellite-containing genomic fragments with short sequence repeat oligonucleotides prior to cloning into phagemid vector (for details, see Ji

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et al. 2003). Recombinant bacterial colonies were manually picked out and cultured in 96-well microtitre plates. They were then transferred onto Hybond-N+ nylon membrane (Amersham), and screened with ^{32}P-labelled oligonucleotide probes following Sambrook *et al*. (1989). Genomic inserts of positive clones were isolated by 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 either ABI PRISM3700 or ABI PRISM3100 automated sequencers.

One hundred and fifty-eight positive clones were obtained after about 800 recombinant bacterial colonies were screened. Sixty-eight of these were sequenced from both strands, and 48 clones were found to contain real microsatellite sequences, with the majority being GT and AG microsatellites (93%). This corresponds to a microsatellite cloning efficiency of 14% in *L. migratoria* (whereas in the cotton bollworm, the value is only 2.5%, Ji *et al*. 2003). However, only 23 sequences were suitable for designing PCR primers. Oligonucleotide primers for amplifying microsatellite loci were designed using OLIGO®4 primer analysis software (National Biosciences Inc.), and synthesized by MWG Biotech-UK or Dingguo Biotech (Beijing).

PCR amplification from genomic DNA template was performed using *Taq* DNA polymerase from Promega on Perkin Elmer GeneAmp 9700 systems. Some 5–10 ng of genomic DNA was used in a 10-μL reaction, with 0.5–1.0 U of *Taq* DNA polymerase, 200 μM of each dNTP, 0.1–0.2 μM of each primer. The 'Touchdown' programming technique (Don *et al*. 1991) was employed for most PCR. Negative control with no DNA template was always carried out along with normal PCR amplification. PCR assays revealed that primers from nine clones appear to amplify repetitive sequences (see also Ji *et al*. 2003), and primers from two clones failed to yield any PCR product. Extensive PCR optimization was carried out for the remaining primer pairs (from 12 clones). Analysis on high resolution Metaphor agarose gel showed that primers from 11 clones produced consistent amplification results, yielding polymorphic bands which appear to be diallelic. One primer from each of the 11 clones was end-labelled with a fluorescent dye (Table 1). After further testing by genotyping about 40 locust individuals in an ABI PRISM3100 automated sequencer with GeneScan-400HD (ROX) as the internal size standard, four more loci were dropped because either heavy stutter bands were observed or they amplified more than two alleles from some individuals. The remaining eight loci show typical characteristics of single-copy diallelic nuclear loci, and were tested in an additional 160 samples. Table 1 lists sequences of these primers, the key experimental parameters for PCR amplification, and some population-genetic estimates. The expected and observed heterozygosities were calculated with the program GENEPOP (Raymond & Rousset 1995). The expected heterozygosities (see Table 1) of these loci range from 0.45 to 0.97 (with seven loci being larger than 0.87), indicating that they are highly polymorphic in natural populations. Various degrees of heterozygote deficiency at these loci were observed in the samples studied. An alternative measure of the variability of microsatellite loci observed in populations is allele

Table 1 Characteristics of eight polymorphic microsatellite loci in *Locusta migratoria*. Primer sequences, repeat unit structure, average expected (H_E) and observed (H_O) heterozygosities, number of alleles, PCR annealing temperatures (T_a) for TouchDown PCR on Perkin Elmer GeneAmp 9700 system and the optimal $MgCl_2$ concentration are indicated for each locus. *Denotes primers that were labelled with fluorescent dyes. GenBank Accession nos: AJ558244–AJ558251

Locus	Primer name	Sequence (5′–3′)	Repeat type	Labellin Dye	T_a (°C)	$MgCl_2$ (mM)	No. of alleles	Size range (bp)	H_E	H_O
LmIOZc9	C9F233	GTAAAATGTATGCCTGCTCATAA	$(GT)_{22}$	NED	57–55	1.5–2.2	26	216–272	0.94	0.86
	C9B455*	TACACATATTCCACAAAACCACTA								
LmIOZc19	C19F6*	ACTCATCAGAAACGTCTGTGACAAA	$(TG)_{16}$	HEX	55–52	1.5	32	240–304	0.96	0.86
	C19B254	TGATAAAATTAAAGGCTGGATGT								
LmIOZc26	C26F686*	AAACATGGCAACAGAGAACAG	$(TG)_{13}T_8$	HEX	55–52	1.25–1.5	45	113–189	0.97	0.88
	C26B788	CACAGCTAGACCCCAAACATA								
LmIOZc29	C29F3*	AGATGGTAGAGCCTTCTTTCTCAT	$(GA)_9GT(GA)_{20}$	HEX	57–55	1.5–2.5	31	220–320	0.95	0.87
	C29B279	CTATCAGTGAGGGCACAACAGTAA								
LmIOZc35	C35F75*	TATGTGCTCCTATAAATCGTGTA	$(GT)_{11}$	FAM	57–55	1.5	21	172–228	0.87	0.72
	C35B228	GATTCTTACCCCGACAGCGATTG								
LmIOZc36	C36F35*	TACAGGGCTCACAGGATCATATT	$(GT)_{21}$	NED	59–55	1.5–2.2	19	191–233	0.88	0.33
	C36B224	TAAGTGCTGCGGTTTTCAGA								
LmIOZc67	C67F1*	ATTTTGCCCAAGAAGATGCCATCA	$(TG)_{20}$	FAM	59–55	1.5–2.2	17	201–239	0.89	0.68
	C67B194	TTCTAGTTGGAGTCTGCAATTATC								
LmIOZc76	C76F33*	TAATGATCTTTCTAAAAACGATAA	$(TG)_3(TTG)_7$	HEX	51–50	1.5	9	116–143	0.45	0.30
	C76B143	GAGATGAAGACGCTTAGGATAG								

Table 2 Cross-amplification of *Locusta migratoria* microsatellite primers in subspecific populations and closely related species

	C9	C19	C26	C29	C35	C36	C67	C76
L. m. migratorioides	+	+	+	+	+	+	+	+
L. m. migratoria	+	+	+	+	+	+	+	+
L. m. tibetensis	+	+	+	+	+	+	+	+
Oedaleus infernalis	–	–	–	–	+	+	–	–
Gastrimargus marmoratus	–	–	–	+	–	–	–	–
Acrida cinerea	–	–	–	–	–	–	–	?
Chondracris rosea	–	–	–	–	–	–	–	–

Note: –, denotes no amplification product or multiple bands obtained; +, denotes specific product (one or two bands) was obtained after PCR optimization; ?, indicates uncertainty on PCR results.

numbers, which vary from nine to 45 in samples genotyped here. It similarly indicates high levels of polymorphism at these loci in populations.

Locusta is a monospecific genus. However, some nine subspecies of *L. migratoria* have been distinguished, with *L. m. migratorioides* occurring in Africa south of the Sahara and offshore Atlantic islands, *L. m. migratoria* in temperate East Europe to North China, Korea and Japan, *L. m. manilensis* in East and South Asia and the Pacific region, and *L. m. tibetensis* in Tibet and nearby West China (Centre for Overseas Pest Research 1982; Guo *et al*. 1991). Microsatellite markers reported here were developed from the oriental subspecies *L. m. manilensis*. Genotyping analysis confirmed their applicability in three other subspecies, that is, *L. m. migratorioides*, *L. m. migratoria* and *L. m. tibetensis* (Table 2). We are therefore confident of their applicability across other conspecific populations.

Cross-species amplification of *L. migratoria* microsatellite markers was investigated in four additional genera of grasshoppers and locusts of the superfamily Acridoidea, viz: *Oedaleus infernalis*, *Gastrimargus marmoratus*, *Acrida cinerea* and *Chondracris rosea* (Table 2). The genera *Locusta*, *Oedaleus* and *Gastrimargus* belong to the same subfamily Oedipodidae (Liu 1990). After extensive PCR optimization and test, it was observed that none of the eight pairs of *Locusta* microsatellite primers can amplify across all four species tested. In fact, no primer pair can specifically amplify in the species tested outside the subfamily Oedipodidae. Only two pairs of primers (LmIOZc35, LmIOZc36) successfully amplified specific products in *O. infernalis*, and only one primer pair (LmIOZc29) succeeded in *G. marmoratus*. In order to verify whether products amplified from other species are microsatellite sequences, PCR product of LmIOZc29 locus amplified from *G. marmoratus* was directly sequenced. This revealed the presence of a perfect GA/TC microsatellite (data not shown). Therefore, microsatellite loci developed here are very *Locusta*-specific, with only very limited cross-species applicability.

A preliminary analysis of population genotyping data also showed that the F_{ST} values of the Tibetan samples and the Hainan samples (from Hainan island in South China) are significantly different from each other and from all other populations, indicating strong genetic differentiation among these populations in spite of the fact that the migratory locusts can carry out long-distance migration. Therefore, these microsatellite loci may be useful polymorphic markers for population genetic analysis.

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