

PRIMER NOTE

PCR primers for polymorphic microsatellite loci in the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae)

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Abstract

Nine pairs of polymerase chain reaction (PCR) primers that amplify polymorphic microsatellite loci in the desert locust, *Schistocerca gregaria* (Forsk.), were developed using a magnetic bead-based enrichment protocol. A sample of 48 locusts collected during the 1993 and 1995 upsurge periods in Eritrea, East Africa, were genotyped. The number of alleles per locus ranged from six to 20; the average was 12.67. Allelic distributions were significantly different between samples from different localities.

Keywords: desert locust, enriched library, microsatellite, *Schistocerca gregaria*

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The desert locust, *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae), is a serious agricultural pest throughout Africa, the Middle East and parts of Asia. It undergoes density-dependent transformation from the solitary phase to the swarming gregarious phase. The solitaria form highly dispersed pocket populations in restricted parts of the species range. The gregaria, characteristic of outbreak populations, are known for their long-distance migration, sometimes across continents, and have the potential to cause huge economic damage (Simpson 1999). The ultimate means of controlling locust plagues may lie in holding locust populations in the recession phase indefinitely. This requires an understanding of the temporal and spatial population dynamics of locusts. Molecular genetic markers provide a useful tool for this purpose. We have developed and fully characterized nine primer pairs that amplify highly variable microsatellite loci.

Our microsatellite enrichment protocol and screening of a desert locust subgenomic library followed a modified version of Hamilton *et al.* (1999) and Glenn *et al.* (2000) as described in Heist *et al.* (2003). Briefly, total genomic DNA was isolated using the DNeasy tissue kit (QIAGEN). DNA from a single individual locust was digested with the restriction enzyme *Mbo*I. Digestion products were electrophoresed in 1.4% agarose TAE gel, and fragments in the 300–1000 bp size range were recovered using the QIAEX II

gel extraction kit (QIAGEN). Subsequently, purified genomic fragments were ligated to complementary linkers (linker F, 5'-CTAAGGCCTTGATCGCAGAAGC-3' and phosphorylated linker R, 5'-P GCTTCTGCGATCAAGGCCTTA-GAAAA-3') as described in Heist *et al.* (2003).

Genomic DNA/linker ligations were hybridized to two biotin-labelled synthetic repeat oligos (GT)₁₅ and (GA)₁₅. A second hybridization was conducted to bind microsatellite-containing genomic DNA/linker/oligo prop to streptavidin MagneSphere paramagnetic particles (Promega). Genomic DNA bound to oligos was eluted by adding 60 µL of pre-heated TE buffer to each sample, heating at 95 °C for 10 min and separating the beads via magnetization. Microsatellite-containing fragments were amplified from the beads via polymerase chain reaction (PCR) using primers complementary to the linkers. The resulting repeat-enriched PCR products were restricted with *Mbo*I and ligated into pUC18 cloning vector, which were then used to transform DH5α competent cells.

PCR-based screening was performed to determine insert length in the positive clones. Products were electrophoresed on a 1.4% agarose gel to visualize insert length. Approximately 84 positive clones with inserts >350 bp were selected for sequencing. Samples were sequenced with BigDye Terminator (Applied Biosystems) cycle sequencing kit using universal M13 primers. Sequences were aligned and edited with SEQUENCHER 4.1 (Gene Codes). PCR primers were designed using the MAC VECTOR DNA analysis software (MAC VECTOR version 6.5, Oxford Molecular) and

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Table 1 PCR primer sequences and polymorphism estimates for nine microsatellite loci of the desert locust, *Schistocerca gregaria* (Forsk.) (1) F: forward, R: reverse primer sequences; (2) annealing temperature; (3) repeat motif in library; (4) length of cloned sequence; (5) total sample size; (6) number of alleles per locus; (7) test of differentiation in allele distribution between the Senhit, Barka and Sahel samples; (8) localities sampled: n = sample size; H_O and H_E are observed and expected numbers of heterozygotes, respectively

Locus	Primer sequence (5'-3') ¹	T_a (°C) ²	Repeat ³	Size ⁴	N^5	N_a^6	P^7	Senhit ⁸		Barka ⁸		Sahel ⁸	
								n	H_O/H_E	n	H_O/H_E	n	H_O/H_E
DL01	F: ACCTTTGGAAAATCTCTGGAGTC R: ATTCCGAATATCGGTAGTGGC	58.5	(CT) ₃₇	228	47	20	0.46	13	9/12.44	17	15/15.88	17	15/16.12
DL03	F: AACTTGTAAATGGACAGCGTGTAGC R: TGCCAGTCGAACAAAAGAATCC	58.5	(GT) ₃₄	134	37	10	0.26	8	2/5.80	13	2/11.96	16	0/7.43
DL06	F: AGGACTGACGCTCCTGGAAGAAG R: CGCCATAAAGTGGTGTGTTC	63	(GT) ₂₀	145	48	17	0.11	13	7/11.48	18	11/16.83	17	12/16.03
DL07	F: AAAAGAGGAAGAAGGCTTGCTG R: GGATGTTACACACAGATTTGAGG	63	(CA) ₂₄	218	48	16	0.03	13	12/11.72	18	13/16.66	17	12/15.73
DL08	F: TCTGGTCACATGATCGTGATG R: TCTGTCTCTCTGTATGCAACG	63	(CA) ₄ A ₂ (CA) ₈	160	44	7	0.19	11	6/6.48	17	12/12.70	16	15/11.52
DL09	F: TTATTGGTGTCCCAGCCTGACC R: CGTCTGCGTATTTTGTCCGAC	61	(GT) ₁₆	200	47	10	0.00	12	9/10.52	18	14/16.06	17	13/15.09
DL13	F: AAGCGAAGTGTGCTGACTGTGC R: GAAACATCATTCGAAATCAGGC	61	(GA) ₃ A ₂ (GA) ₁₈	228	47	13	0.20	12	10/11.13	18	14/16.78	17	11/14.97
DL17	F: ACGGAGAGTGAGAGATAGAGGTGG R: GCATAGGCTTTTCGTGTATTCC	61	(GA) ₁₇	178	48	15	0.16	13	11/12.00	18	8/16.23	17	14/15.88
DL18	F: TTCCAAGACTTACCAAGCGGG R: TTCTGACGAAGCAACTGCCAG	58.5	(CA) ₃ A ₂ (CA) ₆	113	48	6	0.92	13	13/6.76	18	18/11.14	17	17/10.58

subsequently optimized on the Mastercycler gradient thermocycler (Eppendorf).

The utility of microsatellite primers was tested by screening 10 individuals. Genotyping PCR was conducted with a final reaction volume of 10 μ L containing approximately 10 ng of genomic DNA, 0.25 U Thermoprime Plus DNA polymerase (Advanced Biotechnologies), 2.0 mM MgCl₂, 0.2 mM each dNTP, 75 mM Tris-HCl (pH 8.8), 20 mM (NH₄)₂SO₄, and 0.4 μ M each primer. Forward primers were fluorescent labelled with 6-FAM, HEX or NED for visualization. The thermal profile had an initial denaturation at 95 °C for 4 min, followed by 25 cycles at 95 °C for 45 s, annealing temperature (T_a) (see Table 1) for 45 s, 70 °C for 45 s, and a final extension at 70 °C for 4 min. Alleles at individual loci were separated in a 5% denaturing polyacrylamide gel on an ABI 377 using ROX 500 size standard. Table 1 shows the nine primer pairs obtained and some characteristics of the loci they amplified. The clone sequences from which primers were developed have been submitted to GenBank (Accession nos DQ177448–DQ177456).

An outbreak of the desert locust infestation along the Red Sea coast of Eritrea was reported in late 1992. By June 1993, these coastal populations had developed into a serious upsurge (Showler 1995; Ibrahim *et al.* 2000). A sample of 17 locusts from this upsurge collected in the Semhar region of Eritrea, along the Red Sea coast, were genotyped. In the summer of 1995, locust swarms developed much further inland in western Sudan (FAO 1995). The swarms

were believed to have moved eastwards into western Eritrea where local breeding resulted in large nymphal bands in the Senhit and Barka regions. A similar number of locusts from these two regions were also genotyped at the nine microsatellite loci as described previously (Table 1).

Data analysis using GENEPOP on the Web (Raymond & Rousset 1995) followed with the default values for the Markov chain parameters. The number of alleles per locus ranged between 20 in DL01 and six in DL18, the average being 12.67 (Table 1). Tests of differences in the allele frequency distributions at all nine loci between the Senhit and Semhar samples, and the Barka and Semhar samples were statistically significant ($P < 0.01$). In contrast, the comparison between the Barka and Senhit samples was nonsignificant ($P > 0.05$). While the limited sample sizes and the preliminary nature of the study do not allow us to draw conclusive inferences from this trend, we suggest that the Senhit and Barka samples showed similarity because they were from the same 1995 outbreak populations that originated in western Sudan, although collected from different regions in Eritrea 3 weeks apart. With the same caveats in mind, we attribute the distinct allelic make up of the Semhar samples to their origin, a local outbreak along the Red Sea coast in 1993.

Table 1 shows that for most of the loci, the observed number of heterozygotes in the three populations is lower than expected for populations in Hardy–Weinberg equilibrium. The exception is DL18 where, in all three populations,

the observed number of heterozygotes is higher than the expected number. This is also the case in the Sahel sample for DL08. The sample sizes from each of the three populations are too small for a robust test of the statistical significance of these deviations from Hardy–Weinberg proportions. However, global tests as implemented in GENEPOP (option 1, sub-option 4) for each locus with the samples pooled revealed significant heterozygote deficit in all the loci except for DL18 ($P < 0.001$). Thus, it is unlikely that the observed heterozygote deficit is entirely due to presence of null alleles. Multilocus tests for each of the three populations also produced highly significant P values ($P < 0.001$).

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