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PRIMER NOTE

Identification and characterization of 10 polymorphic microsatellite loci in the German cockroach, *Blattella germanica*

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Abstract

Primer sequences and initial characterization are presented for 10 microsatellite loci isolated from the German cockroach, *Blattella germanica*. In a sample of 30 individuals from a single population sample, all loci were polymorphic with two to 12 alleles segregating per locus and levels of observed heterozygosity ranging from 0.27 to 0.92. One locus showed a deficit of heterozygotes. Experimental conditions are described for polymerase chain reaction multiplexing, which enables the genotyping of eight loci in three electrophoretic runs consisting of one set of three and two sets of two markers. Seven primer sets cross-amplify in the related *Blattella asahinai*.

Keywords: *B. asahinai*, *B. germanica*, dinucleotide microsatellite, multiplex PCR, tetranucleotide microsatellite, trinucleotide microsatellite

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Little is known about the population genetics of the German cockroach, *Blattella germanica* (L.). Considered the most important of the household cockroaches (Schal & Hamilton 1990), *B. germanica* pose both direct hazards, as producers of allergens (Rosenstreich *et al*. 1997) and agents involved in the transmission of antibiotic resistant microbes in livestock production systems (Zurek & Schal 2004), and indirect hazards, relating to extensive insecticide use (Schal & Hamilton 1990), to both humans and animals. Contrary to prediction for a species believed to disperse primarily through human-mediated, 'jump' transport, genetic differentiation has been shown to be extremely low among geographically distant populations screened with either allozyme or random amplified polymorphic DNA (RAPD) markers (Cloarec *et al*. 1999; Jobet *et al*. 2000). In contrast *Hin*dIII rDNA fragment polymorphism revealed significant differentiation between populations from geographically proximate locations (10–100 km separation) (Mukha *et al*. unpublished). It is clear therefore that a disparity exists between the information derived from previous studies, possibly a result of the poor resolving power of the markers employed and not actual global panmixia. Microsatellite DNA loci offer an ideal tool for the identification of genetic differentiation across a variety of geographical scales (Avise 2004). Here, we present primer sequences, polymerase chain reaction (PCR) conditions, strategies for multiplex screening, initial characterization of the genetic variation, and preliminary cross-species amplification results for 10 microsatellite markers.

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Enriched DNA libraries were developed following two methods. For the first approach, genomic DNA was extracted from the head and legs of 20 individuals and pooled. Microsatellite enrichment followed the protocol developed by Kijas *et al*. (1994) which utilizes biotinylated oligonucleotides with modifications (details available upon request to P.A. Prodöhl — p.prodohl@qub.ac.uk). Fifty positive clones were sequenced of which 16 contained microsatellite repeats. PCR primers were developed for two loci. The second utilized DNA extracted from the head and thorax of six individuals using the QIAGEN DNeasy Tissue kit and followed the methodology described by Dopman *et al*.

Table 1 Characteristics of 10 microsatellite DNA loci developed for the German cockroach (*Blattella germanica*) and screened for a total of 344 specimens collected in North Carolina, USA: locus designation (GenBank Accession nos: EF029072–EF029081), primer sequences, repeat motif, PCR conditions, sample size (N), number of alleles observed (N_A), average expected (H_E) and observed (H_O) heterozygosities, conformance to Hardy–Weinberg equilibrium (HW test) and range of PCR product sizes in (bp) and cross-species amplification success

					Individuals collected at one location					Individuals collected across 12 locations			Amplification in *Blattella asahinai*		
Locus	Primer sequences	Repeat motif	Annealing temperature (°C)	µM of each primer	N	N_A	H_E	H_O	HW test	N	N_A	Size (bp)	N_A	Size (bp)	GenBank Accession nos
Bg-C04	F: ATTTGCAATACGGCCATCGA R: GGGAATAGAAGAGTGTGACA	(GATA)$_6$	55	3	30	2	0.48	0.27	*	310	4	153–173	4	143–167	EF029074
Bg-G7	F: GCAGTACCATATCCTCAGGA R: CCATGTTAGGTACCTTCAGA	(GTTA)$_8$	55	3	30	6	0.76	0.7	ns	343	8	214–242	6	219–250	EF029075
Bg-B12	F: GTCCAGCATGTTTTGCA R: GACATTTAGGAGAGTGGTCA	(ATAG)$_{14}$ACAG(ATAG)$_3$ CCAG(ATAG)$_2$CCAGACAA (ATAG)$_3$TAT(ATAG)$_3$	55	3	30	10	0.63	0.63	ns	342	15	334–392	na		EF029076
Bg-D05	F: AGGATGACGTCAAGGAACAA F: GGAATTGTGGCATAGCA	(ATAC)$_{15}$	55	1.5	25	9	0.83	0.92	ns	328	15	146–230	3	119–215	EF029077
Bg-1D5	F: AATCACGAGTTGCCTACCAA R: TTCAAGGTCCTCAACAGTCA	(GA)$_{18}$	55	1.5	24	12	0.83	0.75	ns	323	22	205–267	na		EF029078
Bg-D9	F: GGATCTGTGGATATCCCCTA R: TACAAGAGCGATGAGTCTCA	(GAA)$_{20}$	55	2	29	7	0.81	0.76	ns	319	21	206–279	na		EF029080
Bg-A7	F: AGCAACCTGTTAGGGGAGGA R: TGTGGCACGGATGGGAGA	(AACT)$_7$	55	2	29	4	0.55	0.66	ns	316	8	301–349	4	297–305	EF029081
Bg-F7	F: TACCGGTTAGAGCGGGTGA R: ATCAAATTGGTCGCAGCGTA	(GATT)$_{12}$	55	2	27	6	0.74	0.81	ns	323	11	194–222	31	99–219	EF029079
Bg-wb-1 A	F: GATGCAGATTCTTGATTGA R: TACATTGACGGCTAACCCTCTA	(GAA)$_{10}$	60	1.5	26	4	0.47	0.46	ns	293	9	180–218	5	167–197	EF029072
Bg-wb-2 A	F: GACAATGGGAGAAATGAGGAGAA R: GGGCCGATAGCGAGTGG	(GATA)$_4$AATA(GATA)$_2$ AATA(GATA)$_3$	55	1.5	30	4	0.65	0.57	ns	332	6	258–282	3	278–286	EF029073

HW test, Hardy–Weinberg test (Raymond & Rousset 1995) — *significant probability test ($P < 0.05$); ns, nonsignificant. Cross-species amplification — na, nonamplification.

(2004). Overall, 112 positive clones were sequenced and PCR primer pairs were designed for 16 loci.

Primer pairs were optimized using 30 individual adult male *B. germanica* collected within a swine farm in Garland County, North Carolina, USA. Genomic DNA was extracted from samples using the PUREGENE DNA isolation kit (Gentra Systems). PCRs were carried out in 12-μL volumes, each containing 1× PCR buffer, 2.5 mM $MgCl_2$, 100 μM dNTPs, 50 ng DNA template, 0.5 U *Taq* DNA Polymerase (Bioline), and ddH_2O to 12 μL. Primer concentration varied between 1.5 and 3 pmol with the forward primer of each end-labelled with an M13F-29/IRD700 IRDye tag (Li-Cor, Inc). Annealing temperatures ranged from 55 to 60 °C (see Table 1 for details). PCR cycling conditions were comprised of an initial denaturation stage of 5 min at 95 °C, followed by 27 cycles each consisting of 1 min at 95 °C, 1 min at the ideal temperature for each primer set, and 1 min at 72 °C carried out using PTC-100 thermal cyclers (MJ Research, Inc). Following PCR, 5 μL of stop solution (95% formamide, 20 mM EDTA, bromophenol blue) was added to each reaction. Reactions were subsequently denatured at 90 °C for 4 min and loaded onto 25 cm 6% 1× TBE polyacrylamide gels, run on a Li-Cor 4300 automated DNA sequencer, and sized using 50–350 bp IRDye700 standard (Li-Cor, Inc). Gels were run at a constant power of 40 W at 50 °C for 2 h. Results were analysed using GENEPROFILER software (Scanalytics, Inc).

Ten primer pairs produced unambiguous PCR products demonstrating allelic variation (Table 1). All loci were polymorphic in *B. germanica*, with four to 22 alleles per locus observed across 12 populations. Observed heterozygosities ranged from 0.27 to 0.92. No evidence for linkage disequilibrium (GENEPOP, version 3.3; Raymond & Rousset 1995) was detected among the 45 possible pairwise locus comparisons. Tests for conformance to Hardy–Weinberg equilibrium (HWE) (GENEPOP) indicated a significant deviation from expectations at one locus (Bg-C04). A heterozygote deficiency test (GENEPOP) performed on this locus revealed a significant heterozygote deficit ($P = 0.016$) indicating possible occurrence of null alleles. Three multiplex sets were possible: set one — Bg-C04, Bg-G7, Bg-B12; set two — Bg-D05, Bg-1D5; and set three — Bg-D9, Bg-A7. PCR conditions followed those outlined above. Cross-species amplifications were tested on five individuals of each of five cockroach species: *Periplaneta americana* and *Periplaneta fuliginosa* (family Blattidae), *Parcoblatta pennsylvanica*, *Supella longipalpa* and *Blattella asahinai* (family Blattellidae). Successful locus amplification was only observed for *B. asahinai* for seven of the 10 loci tested.

Preliminary results suggest these markers yield sufficient within and between population polymorphism for the resolution of patterns of dispersal and gene flow among *B. germanica* populations.

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