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PRIMER NOTE

Polymorphic microsatellite loci in the mosquito *Aedes polynesiensis*

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Abstract

The *Aedes scutellaris* complex of mosquitoes contains the most important vectors of lymphatic filariasis in the South Pacific region, particularly *Aedes polynesiensis*. Six microsatellite loci were isolated and characterized from this species; all were polymorphic and appear to be useful markers for population studies. Five of the primer pairs also amplified homologous products from three other species in the *A. scutellaris* group and from the important dengue vector *Aedes albopictus*.

Keywords: *Aedes*, albopictus, filariasis, microsatellite, mosquito, scutellaris

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Aedes polynesiensis Marks is the most widespread and important member of the *Aedes scutellaris* complex of mosquitoes, ranging from Fiji to the Tuamotu Archipelago. In this region of the South Pacific it is the main vector of subperiodic Bancroftian filariasis (caused by *Wuchereria bancrofti*), and is also able to transmit dengue virus and Ross River fever. Control using insecticides has proven difficult, as the adults rest outdoors, and the larvae utilize a wide variety of natural habitats. Island populations are well suited to genetic control techniques, however, and Macdonald (1976) first suggested genetic replacement to render populations unable to transmit filariasis. *A. scutellaris* species from the western South Pacific region into Southeast Asia are fully *Wuchereria*-refractory and many can be crossed successfully with *A. polynesiensis* (Meek & Macdonald 1982). The fragmented island population structure of *A. polynesiensis* also make it a good subject for studies on evolutionary divergence.

Genetic markers with high levels of polymorphism are needed in *A. polynesiensis* for studies to estimate degrees of genetic differentiation (Failloux *et al*. 1997), migration rates, geographical origins of island populations, and for the genetic mapping of filarial susceptibility. With their high mutation rates, codominant inheritance, and abundant distribution across the genome, microsatellites have proven to be highly suitable markers in numerous population/genetic mapping studies in other mosquito groups. We therefore undertook microsatellite isolation in *Aedes polynesiensis* using a hybridization enrichment procedure (Bloor *et al*. 2001).

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Total *A. polynesiensis* genomic DNA was extracted from six whole mosquitoes, using recently colonized material from Fiji, following the protocol of Collins *et al*. (1987). Pooled DNA was partially digested with *Sau*3AI for 4 h at 37 °C. A double-stranded adaptor with an overhang complementary to *Sau*3AI-digested DNA was prepared by mixing 1.5 nmol of each of two oligonucleotides S61 5′-GGCCAGAGACCCCAAGCTTCG-3′ and the 5′-phosphorylated S62 5′-GATCCGAAGCTTGGGGTCTCTGGCC-3′ in equal volumes, heating the solution to 80 °C for 5 min and allowing it to cool at room temperature for 1 h. The adaptor was then ligated to the digested fragments, the product run in a 1.5% agarose gel, and fragments between 500 and 1000 bp excised and purified using QIAGEN columns.

To capture trinucleotide microsatellite-containing DNA fragments, 100 µL of streptavidin-coated magnetic beads (10 mg/mL) (Dynabeads®; Dynal) were washed and attached to 200 pmol of biotinylated oligonucleotides 5′-$(CCA)_{10}$GCCACT-3′-biotin and 5′-$(TGA)_{10}$GCCACT-3′-biotin (SIGMA-GENOSYS). The oligonucleotides were then hybridized to the size-selected DNA for 1 h at 60 °C. Following differential stringency washes in 2× and 1× saline sodium citrate buffer (SSC) and two washes at 60 °C and 65 °C in 1 × SSC, the enriched DNA was recovered, made double-stranded by polymerase chain reaction (PCR) and ligated into the pGEM-T plasmid (Promega). Plasmids were cloned into JM109 competent cells (Promega), selected

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Table 1 Characterization of six microsatellites for 30 wild-caught *Aedes polynesiensis*. N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. The forward primers were labelled with Beckman dyes D2 (*Ap1*F, *Ap2*F), D3 (*Ap3*F, *Ap4*F) and D4 (*Ap5*, *Ap6*). GenBank Accession nos for clone sequences *Ap1–Ap6* are AY438532–AY438537

Locus	Primer sequence (5′– 3′)	Repeat motif	Cloned allele size (bp)	Allele size range (bp)	N_a	H_O (H_E)
Ap1	F: GCACCAGAGCAAAAGTAGCC R: GGGAAGAGAAAGAAGCACCC	$(AC)_{14}N_3(CG)_5$	129	(108–138)	12	0.633 (0.886)
Ap2	F: ATTACCGCCGTACTGCTGAG R: CATCACCACCATCACCAAAC	$(TGC)_{12}N_6(TGA)_4$	148	(122–155)	11	0.767 (0.817)
Ap3	F: AGGAGTGTTCTGCTGTTGGG R: GCAAACTTTTCCCTTCCTCC	$(TGC)_5$	111	(97–109)	4	0.167 (0.295)
Ap4	F: CCACAAAAAGCCAAAAGAGC R: ACTTGGGAGTGATGGTGTGG	$(TCA)_6$	151	(147–156)	4	0.433 (0.556)
Ap5	F: AGATGGTGCTGGGTGAAGAC R: AGTGCAAACAACACCAGCAG	$(TGG)_4N_{30}(TGC)_4(TGT)_3N_{12}(TGA)_3$	144	(132–147)	4	0.133 (0.159)
Ap6	F: CTACTCTGTAGACCATGGCGG R: TCAGCGGAGAGTTGATGTCC	$(CT)_3N_{26}(CAC)_3N_{36}(CAC)_3(CAG)_4(CA)_3$	186	(175–191)	5	0.167 (0.247)

Table 2 Microsatellite PCR product sizes (bp) in other members of the *Aedes scutellaris* species complex and in *Aedes albopictus*. Sample sizes are shown in parentheses, and for *A. albopictus* the results of sequencing each locus from one homozygous individual are also shown; GenBank Accession nos for these sequences are AY438538–AY438542

Locus	*A. polynesiensis* clone size	*A. albopictus* with sequence repeat motif	*A. katherinensis*	*A. pseudoscutellaris*	*A. tongae*
Ap1	129	104–106 (3) $(AC)_5N(CG)_3$	104–106 (3)	110–114 (2)	92 (1)
Ap2	148	118–124 (3) $(TGC)_5N_6(TGA)_4$	114 (2)	114–121 (5)	118–122 (2)
Ap3	111	97–101 (3) $(TGC)_3$	—	98–107 (5)	98–107 (3)
Ap5	144	143–146 (3) $(TGC)_3N_6(TGT)_1N_{12}(TGA)_3$	143 (2)	146 (5)	140–143 (3)
Ap6	186	182 (3) $(CAC)_3N_{33}(CAG)_3N_3(CA)_3$	180–184 (2)	185–191 (4)	168–173 (2)

and purified with Qiaprep columns (Qiagen) and 22 inserts of 300–1000 bp sequenced using Universal and Reverse primers. NCBI BLAST searches showed no homologies to GenBank sequences.

Six loci containing microsatellite motifs were used for the design of PCR primers in the regions flanking the microsatellite sequence, using PRIMER3 software (Whitehead Institute/MIT Center for Genome Research), in order to ensure similar optimal annealing temperatures and products of 100–200 bp. PCR was carried out in a final volume of 15 μL containing 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.2 mM of each dNTP, 0.75 μM of each primer, 0.75 U *Taq* (Sigma) and 1 μL of genomic DNA extracted from whole single mosquitoes as above and re-suspended in 100 μL water. The cycling conditions on Techne Progene thermal cyclers were as follows: 5 min denaturation at 94 °C followed by 35 cycles of 94 °C for 25 s, 50 °C annealing (T_a) for 28 s and 72 °C for 30 s, with a final extension step of 5 min at 72 °C. PCR products were sized on a Beckman Coulter Ceq8000 automated sequencer, using the manufacturer's fragment detection chemistry.

The six loci were characterized for degree of polymorphism, using collected specimens of *A. polynesiensis* from Sigatoka and Suva in Fiji. The results of screening 30 individuals (15 from each location) at six loci are shown in Table 1. All the loci are polymorphic, showing easily scored alleles; despite the compound interrupted repeat motif structure of some of the microsatellite loci they appear well suited to population genetic studies in *A. polynesiensis*. Tests carried out using GENEPOP software for Hardy–Weinberg equilibrium for each locus at the two sites showed no significant departures, and none of the pairwise tests for linkage disequilibrium was significant, although the sample sizes were small.

Other workers have reported a scarcity of microsatellite loci in *Aedes aegypti* (e.g. Fagerberg *et al*. 2001; D. Severson, personal communication), so they are either more abundant in *A. polynesiensis* or the particular enrichment procedure employed may have circumvented this problem. Three other species in the *A. scutellaris* complex were also assayed: *A. katherinensis*, a filariasis-refractory species from Northern Australia (colony material), and field-collected specimens of the filariasis-susceptible species *A. tongae* from Tonga and *A. pseudoscutellaris* from Fiji. In most cases alleles in the anticipated size range were amplified at five of the loci (Table 2). In addition, three individuals were assayed from a colony (origin Singapore) of *A. albopictus*, which is not a member of the *A. scutellaris* species complex but is closely related, having been included in the *scutellaris* subgroup of the Stegomyia (Belkin 1962). PCR products of the correct size were amplified for five of the six loci. Sequencing of these PCR products from a homozygous individual for each locus confirmed that *Ap1*, *Ap2*, *Ap3* and *Ap5* contained exactly the same microsatellite motifs in *A. albopictus* as for *A. polynesiensis*, while the *Ap6* motif was very similar (Table 2). These five microsatellites would also appear to be useful markers for *A. albopictus*, a major vector of dengue fever globally, where population markers are important for the study of its continuing range expansion and invasion of new territories.

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