

PERMANENT GENETIC RESOURCES NOTE

Eight microsatellite loci for the sexually transmitted, parasitic mite *Coccipolipus hippodamiae*

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Seven dinucleotide and one trinucleotide polymorphic microsatellite loci were isolated from the mite *Coccipolipus hippodamiae*. This mite is an ectoparasite of coccinellid beetles (ladybirds), principally the European two-spot ladybird *Adalia bipunctata*, where it causes sterility in the female host. Levels of genetic diversity were assessed using 32 mites from Warsaw, Poland. We observed moderate variability, with the number of alleles per locus varying between 2 and 4, and observed and expected heterozygosities ranging from 0.031 to 0.267 and between 0.062 and 0.526, respectively. This is the first description of microsatellite loci from the genus *Coccipolipus* and these loci are currently being employed to answer fundamental questions about the epidemiology of *C. hippodamiae* infections on *A. bipunctata*.

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Sexually transmitted infections (STIs) affect many species of vertebrates and invertebrates, often producing high morbidity, and therefore, strong selection on their host (Lockhart *et al.* 1996). One of the best studied invertebrate STI model systems is the ectoparasitic mite *Coccipolipus hippodamiae* (Tarsonemoidae: Podopolipidae) and its primary host the European two-spot ladybird *Adalia bipunctata* (Coleoptera: Coccinellidae) (Webberley *et al.* 2004, 2006). The mite is an important associate of this beetle (and also other species of European coccinellid beetle), achieving high prevalence and causing sterility of the infected female ladybird host within 10–15 days of infection. We developed a panel of polymorphic microsatellite loci for *C. hippodamiae* to investigate this species' epidemiology.

Total genomic DNA for library construction was isolated from adult *C. hippodamiae* using a standard high salt method after an incubation (at 50 °C for ~16 h at 37 °C) with proteinase K. To construct the partial genomic library, approximately 9 µg of pooled DNA was digested using 40 U *Sau3A* restriction enzyme (Boehringer-Mannheim) and then ligated to 50 pM of phosphorylated linkers (*Sau3A* 5'-GGCCAGAGACCCCAAGCTTCG-3' annealed to *Sau3A* 5' CCGTCTCTGGGGTTCGAAGC 3'; (Refseth *et al.* 1997)

using 40 U T4 DNA ligase (Promega). DNA fragments between 400–1200 bp were excised from a 2% agarose gel and purified using a QIAquick gel extraction kit (QIAGEN). Full details of the enrichment procedure (based on Gardner *et al.* 1999) are provided elsewhere (Bloor *et al.* 2001). Briefly, we hybridized the DNA fragments with M2-80 streptavidin-coated magnetic beads (Dynal) that had been incubated with 3'-biotin-labelled (CA)₁₂ and (CAA)₈ oligonucleotides. After a series of differential stringency washes in 2 × SSC and 1 × SSC, the enriched DNA was amplified in a 50-µL PCR primed with 30 pmol *Sau3A*, 75 mM Tris-HCl (pH 8.8), 20 mM (NH₄)₂SO₄, 0.01% (v/v) Tween 20, 0.2 mM each dNTP, 1.5 mM MgCl₂, 1.25U DNA polymerase (ABgene); PCR conditions were: 95 °C for 3 min, 6 × (95 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s), 25 × (92 °C for 30 s, 55 °C for 45 s, 72 °C for 55 s), 72 °C for 10 min. The DNA was purified using a QIAquick PCR purification kit (QIAGEN), ligated into pGEM-T vector (Promega) and transformed into JM109-competent cells (Promega). Recombinant clones were identified using black/white screening on S-gal (Sigma) agar/ampicillin plates. Plasmids containing a microsatellite insert were identified by two or more amplified products after PCR primed with 10 pmol *Sau3A* oligonucleotide and 10 pmol of microsatellite oligonucleotide (CA₁₂). Positive clones were cycle-sequenced using Big Dye chemistry and electrophoresis

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Table 1 Levels of variability at eight microsatellite loci in the ectoparasitic mite *Coccipolipus hippodamiae* from Warsaw, Poland ($n = 32$). N_a indicates number of alleles observed; H_O , observed heterozygosity; H_E , expected heterozygosity; *indicates significant departure ($P < 0.05$) from expected Hardy–Weinberg equilibrium conditions

Locus	Primer sequence (5'–3')	Repeat motif	Size range† (bp)	N_a	H_O H_E
<i>Cohi001</i>	F: GGTTTGCTTTTGGAGAATAGAAGAG BV729462 R: CAAGCTTCGTATCTAAATGTGTG	(TG) ₁₀ (TA) ₃ (GT) ₉	262–274	3	0.161 0.302
<i>Cohi002</i>	F: ATTGGCAGCTTGAGATTG BV729463 R: AATCATCACCACCAACAG	(GTT) ₉ ... (GTT) ₃	154–192	3	0.063 0.062
<i>Cohi003</i>	F: AAGACAGGAAGATGGTCAGTTTG BV729464 R: GAITCAACAGTTGTTCCAC	(CACATA) ₇ (CA) ₈	175–187	4	0.267 0.397
<i>Cohi004</i>	F: TGATTCTTTTACTTTGCTTCATC BV729465 R: TTTTCTAAACGCCTCCTTCC	(TG) ₆ ... (TG) ₁₁ ... (TG) ₁₆	178–196	2	0.031* 0.289
<i>Cohi005</i>	F: AATAGAATGGTGGTAGCGTGTG BV729466 R: GGCGTTGATCTAATCCATCTTG	(CA) ₁₇	280–284	3	0.094* 0.526
<i>Cohi006</i>	F: AATGGTGGTAGCGTGTGC BV729467 R: GTTTGGTATTTATTGCAGATTG	(CA) ₁₈	245–249	3	0.188* 0.454
<i>Cohi007</i>	F: GATCAAATGTGTGACGAGTATG BV729468 R: CATTTGCTTGGCTTTGTGTG	(TG) ₃₇	336–380	3	0.063* 0.404
<i>Cohi008</i>	F: TTTGTGCATTTGTGTATTTG BV729469 R: GCTTCGGATCCAATTACCAG	(TG) ₆ (TG) ₆ (TG) ₃	143–163	2	0.067 0.131

†size of alleles without tail.

on an ABI3130xl (Applied Biosystems). Primers flanking microsatellite regions were designed using Primer 3 (Rozen & Skaletsky 2000).

Microsatellite alleles were amplified in a 10 µL PCR on a Dyad DNA Engine (MJ Research Inc.) using a tailed primer method, whereby forward primers are synthesized with a 5' (or tail) sequence of a third primer that is labelled with either 6-FAM, NED, PET or VIC fluorophores (Applied Biosystems) and which is used to label the PCR products (see (Schuelke 2000)). The PCR consisted of 75 mM Tris-HCl (pH 8.8), 20 mM (NH₄)₂SO₄, 0.01% (v/v) Tween 20, 0.2 mM each dNTP, 3 mM MgCl₂, 5–50 ng template DNA, 3 pmol of tailed, reverse and labelling primer, 10 µg BSA, 1.25 U DNA polymerase (Abgene). PCR conditions for all loci were 95 °C for 3 min, 6× (95 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s), 25× (92 °C for 30 s, 53 °C for 45 s, 72 °C for 55 s), 72 °C for 10 mins. PCR products were pooled with a 500-bp (LIZ) size standard (Applied Biosystems), separated by capillary electrophoresis on an ABI3130xl and sized using GeneMapper software (Applied Biosystems). The online version (3.4, <http://wbiomed.curtin.edu.au/genepop/>) of GenePop (Raymond & Rousset 1995) was used to calculate basic measures of genetic diversity, the significance of any deviations from expected Hardy–Weinberg equilibrium (HWE) conditions and also for linkage disequilibrium between all pairs of loci.

We isolated and sequenced 41 clones with repetitive regions but were able to design primers around 20 loci only because of insufficient or unsuitable sequence flanking the microsatellite locus. Twelve loci were dropped sub-

sequently due to unreliable PCR amplification or because they amplified spurious bands. None of the eight remaining loci amplified *A. bipunctata* DNA, thus ensuring that these microsatellites were derived from the *C. hippodamiae* genome, and they were tested for variability in 32 mites that had infected *A. bipunctata* from Warsaw, Poland (52°13'48.44"N, 21°00'42.71"E). All eight loci produced PCR products in the expected size range. They were moderately polymorphic, resolving between 2 and 4 distinct alleles per locus and with observed and expected heterozygosities that varied between 0.031 and 0.267, and 0.062 and 0.526, respectively (Table 1). Four loci (*Cohi004*, *Cohi005*, *Cohi006* and *Cohi007*) had significant heterozygote deficits from expected HWE conditions, which is most likely a consequence of asexual reproduction in *C. hippodamiae*. No pairs of loci showed significant linkage disequilibrium ($P > 0.05$, after sequential Bonferroni correction for $k = 28$ tests) (Rice 1989).

These microsatellite loci are being used to determine the epidemiological structure of *C. hippodamiae* infections in populations across Europe. Moreover, these loci are probably good candidates for testing in related species such as *Coccipolipus macfarlanei*, another sexually transmitted parasite of coccinellid beetles of which little biology is known.

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