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Nicholas Miller
*University of Nebraska-Lincoln*, nmiller11@iit.edu

A. J. Birley
*University of Birmingham*

G. M. Tatchell
*Horticulture Research International, Wellesbourne, UK*, mark.tatchell@hri.ac.uk

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# Polymorphic microsatellite loci from the lettuce root aphid, *Pemphigus bursarius*

N. J. Miller,[1,2] A. J. Birley,[2] and G. M. Tatchell [1]

1. Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK,
2. School of Biosciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

*Corresponding author* – G. M. Tatchell, fax 44 1789 470552, email mark.tatchell@hri.ac.uk

*Present address for A. J. Birley* – Queensland Institute of Medical Research, The Bancroft Centre, 300 Herston Rd, Herston 4006, Queensland, Australia.

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*Pemphigus bursarius* is an occasional pest of agriculture with an annual life cycle that alternates between sexual reproduction on black poplars (*Populus nigra*) and parthenogenetic reproduction on the root systems of various Compositae, including lettuce. In general, aphids exhibit low allozyme variability (e.g. Tomiuk & Wöhrmann 1980), possibly as a consequence of the response of parthenogenetic lineages to natural selection (Hales *et al*. 1997). Microsatellite loci are typically more variable than allozymes (Tautz 1989) and are likely to provide a more informative system for the study of aphid populations.

A microsatellite-enriched *P. bursarius* genomic library was constructed according to the methods of Edwards *et al*. (1996). Simultaneous enrichment was carried out for a variety of microsatellite motifs by using oligonucleotides with the following sequences: $(AT)_{15}$, $(GT)_{15}$, $(GA)_{15}$, $(GC)_{15}$, $(GCC)_{10}$, $(CAA)_{10}$, $(ATT)_{10}$, $(CATA)_{10}$, and $(ATAG)_{10}$. Enriched DNA was digested with *Mlu*I, cloned into the *Bss*HII site of pJV1 (Edwards *et al*. 1996) and used to transform *E. coli* DH10B (Life Technologies). Plasmid DNA was extracted from 96 positive clones, digested with *Xho*I and separated on agarose gels to determine the size of the cloned fragment. Thirty-six clones with inserts >100 bp were chosen at random for sequencing. Clones were sequenced using a Big Dye dye-terminator kit (Applied Biosystems), following the manufacturer's instructions, with pUC19 universal forward primer. Sequences were detected using an ABI 377 DNA sequencer (Applied Biosystems).

Genomic DNA was extracted from 27 *P. bursarius* fundatrices (stem mothers, foundresses) collected from Lombardy poplars (*P. nigra* var. *italica*) growing at Horticulture Research International using a Puregene kit (Gentra Systems) according to the manufacturer's instructions. The use of fundatrices as a source of DNA ensured that all individuals were from different clones. Microsatellite loci were amplified in a 20 µL PCR reaction containing 10–50 ng of DNA, 1 unit of *Taq* DNA polymerase (Boehringer), 200 µM each dNTP, 0.25 µM each primer, 10 mM Tris, 50 mM KCl and 2.0–3.5 mM $MgCl_2$, overlaid with 30 µL of mineral oil (Sigma). Amplification was carried out in a Hybaid TR1 thermal cycler using a cycle programme of 3 min at 95 °C followed by 35 cycles of 95 °C for 1 min, the primer annealing temperature for 1 min and 72 °C for 1 min, followed by a final incubation at 72 °C for 30 min. The forward member of each primer pair was labelled at the 5′ end with one of the fluorescent dyes 6-FAM, TET, or HEX by the supplier (Genosys). Amplified loci were separated through 4% polyacrylamide gels at 3 kV for approximately 1 h in an ABI 377 DNA sequencer. Amplified fragment sizes were estimated by comparison to TAMRA-labelled GS350 size standards (Applied Biosystems) co-loaded with each sample, using GENESCAN 2.1 (Applied Biosystems). Unbiased allele frequencies and expected heterozygosities ($H_E$) were calculated using the GENEPOP 3.1 computer program (Raymond & Rousset 1995).

Sequencing of 36 clones yielded 32 different sequences, 15 of which (50%) contained a microsatellite. Although enrichment was carried out for several repeat motifs, the majority of cloned microsatellites had a $(GCC)_n/(CCG)_n$ motif. It is unclear whether this is an artifact of the enrichment process or reflects the distribution of repeat types in the *P. bursarius* genome. Primers were designed to amplify seven of the microsatellites and each primer pair was tested at annealing temperatures ranging from 50 to 64 °C in 2 °C increments and at $MgCl_2$ concentrations from 0.5 to 4.0 mM in 0.5 mM in-

**Table 1.** Properties of five *Pemphigus bursarius* microsatellites. Variation was assessed on a sample of 27 individuals collected from a single stand of Lombardy poplar growing in Warwickshire, UK

| Locus | Cloned repeat | Primer sequences (5′→ 3′) | GenBank accession no. | $T_a$ (°C) | [$MgCl_2$] (mM) | Size range (bp) | Number of alleles | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|---|
| Pb 02 | $(CCG)_7$ | HEX-ATTCAGACCGTCCGGCGTTC<br>TGGCAGTCCGTCTCGACTTG | AF267192 | 62 | 2.0 | 84–96 | 4 | 0.44 | 0.55 |
| Pb 10 | $(CGG)_7$ | 6FAM-CTCTCGGGAGGGATTTGA<br>GTAACGCCACGCCAAGAT | AF267193 | 58 | 3.0 | 104–110 | 3 | 0.19 | 0.17 |
| Pb 16 | $(GGC)_6$ | HEX-CTGGTCGTGTAGTAAGTC<br>AACGCTAACTCCTCTGTC | AF267194 | 50 | 3.5 | 150–168 | 5 | 0.23 | 0.37 |
| Pb 23 | $(GGC)_5$ | TET-GACAGACTTCGGTATGTG<br>ACTGCCAACACCGTCACT | AF267195 | 54 | 3.0 | 92–110 | 7 | 0.89 | 0.79 |
| Pb 29 | $(GCC)_5ACC(GCC)_2$ | 6FAM-TTTAACGGACGGCCATTG<br>CGTAGAGACCGAAGGTGA | AF267196 | 54 | 3.5 | 182–194 | 5 | 0.54 | 0.81 |

crements. Five primer sets successfully amplified the target locus at their optimum annealing temperatures and $MgCl_2$ concentrations. However, primer set Pb 29 required the inclusion of a proprietary product, 'Q solution' (Qiagen), at 1× concentration in the PCR reaction to suppress the amplification of non-target sequences.

The variability of the five loci in a population sample is summarized in Table 1. All were found to be polymorphic, with 3–7 alleles and observed heterozygosities ($H_O$) ranging from 0.19 to 0.89. These markers are currently being employed to investigate aspects of population structure and aphid–host plant relationships in *P. bursarius*.

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