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

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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*III 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 manufac-

turer'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 MgCl₂, 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 MgCl₂ 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 TGGCAGTCCGTCTCGACTTG	AF267192	62	2.0	84–96	4	0.44	0.55
Pb 10	(CGG) ₇	6FAM-CTCTCGGGAGGGATTGA GTAACGCCACGCCAAGAT	AF267193	58	3.0	104–110	3	0.19	0.17
Pb 16	(GGC) ₆	HEX-CTGGTCGTGTAGTAAGTC AACGCTAACTCCTCTGTC	AF267194	50	3.5	150–168	5	0.23	0.37
Pb 23	(GGC) ₅	TET-GACAGACTTCGGTATGTG ACTGCCAACACCGTCACT	AF267195	54	3.0	92–110	7	0.89	0.79
Pb 29	(GCC) ₅ ACC(GCC) ₂	6FAM-TTTAACGGACGGCCATTG 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 MgCl₂ 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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