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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite loci from the woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae: Eriosomatinae)

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Abstract

Eight novel microsatellite primer pairs are presented for *Eriosoma lanigerum*, representing the first microsatellite markers available for this genus. Loci were characterized for 27 individuals from one single orchard in Central Chile. All loci were polymorphic within *E. lanigerum* (three to 11 alleles per locus; observed heterozygosity ranging from 0.41 to 0.93), and are therefore useful for population genetic studies within the species.

Keywords: apple pest, Chile, *Eriosoma*, Eriosomatinae, microsatellite, population genetics

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The woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) is an important pest of apple orchards worldwide (Blommers 1994). In North America, this aphid species is reported to reproduce by cyclic parthenogenesis (i.e. alternating one round of sexual reproduction with several rounds of parthenogenesis), with sexual forms mating on elm (*Ulmus americana* L.) and asexual forms multiplying on apple trees (*Malus domestica* Borkh) (Blackman & Eastop 1994). In other geographical regions, this aphid reproduces predominantly through obligate parthenogenesis, with year-round asexual forms occurring on apple trees (Blackman *et al*. 1994; Sandanayaka & Bus 2005; Timm *et al*. 2005). Production of sexual forms in areas with mild winters and the absence of the primary host have been reported (Sandanayaka, Bus 2005). However, the genetic contribution of sexual genotypes to asexual populations infesting apple trees has not been clarified (Sandanayaka, Bus 2005; Timm *et al*. 2005). In Chile, this species occurs in all the apple production area (from 33° to 37° south latitude). The isolation of microsatellite markers would contribute to assess the relative importance of sexual reproduction vs. clonal spread, and assist in the study of the population genetic structure of this important insect pest.

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Genomic DNA was extracted from a single *E. lanigerum* individual following the 'salting out' protocol described by Sunnucks & Hales (1996). Three separate libraries were cloned by ATG Genetics (Vancouver, BC, Canada) following Khasa *et al*. (2000): (i) *Hae*III/PsHA1 restriction/T4 DNA ligation to linker M28/M29p (M28 5'CTCTTGCTTGAATTCGGACTA, M29p 5' pTAGTCCGAATTCAAGCAAGAGCACA); (ii) *Rsa*I/PsHA1 restriction/T4 DNA ligation to linker M28/M29p; and (iii) *Alu*I/PsHA1 restriction/T4 DNA ligation to linker M28/M29p. After digesting with *Eco*RI, fragments were ligated to a dephosphorylated *Eco*RI-treated pGEM3Z+ (AMPR) and electroporated into *Escherichia coli* DH10B. All three libraries were enriched for AG_{17}, TG_{24}, AAG/C_{21}, $GATA_{24}$, and $CATA_{24}$ motifs. Then, bacterial clones were amplified by polymerase chain reaction (Khasa *et al*. 2000), and the amplicons sequenced using BigDye Terminator version 3.1 Sequencing Standard Kit in an ABI 3730 sequencer (Applied Biosystems), using M13 forward and reverse primers (Sigma Genosys Canada). Of 36 successfully sequenced clones, 18 contained repeats motifs (di, tri and tetranucleotide). Primers were designed for all the positive clones either manually or using Primer 3 (Rozen & Skaletsky 2000). To evaluate the polymorphism and reproducibility of the 18 set of primers designed, and to set up the polymerase chain reaction (PCR) conditions, 27 *E. lanigerum* individuals from a single orchard in the central valley of Chile (33° to

Table 1 Characteristics of eight microsatellite loci cloned from *Eriosoma lanigerum*, described by locus name, repeat motif, GenBank Accession number, and oligonucleotide primer sequences. All individuals tested amplified successfully for each locus. Additional information for the whole data set (n = 27) is reported as allele size range, optimal annealing temperature (T_a), number of alleles observed (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E) and significance of departure from Hardy–Weinberg equilibrium (HWE),* loci that significantly deviated from HWE

Locus	Repeat	GenBank Accession no.	Primer sequences (5′–3′)	Size range (bp)	T_a (°C)	N_A	H_O	H_E	HWE P value	No. of individuals successfully genotyped
Erio3	$(TC)_9(CTAT)_6$	EU410510	F: GCCAAACAGTCTTATCTTTCC R: GAATTCGCTGGCTCTCTCTCT	147–163	60	5	0.63	0.56	0.038*	27
Erio20	$(CAA)_{10}$	EU410511	F: CGACCTTGAGCCTTTGAAAC R: CTGGCTCACTTCCTGGTAGC	161–179	59	7	0.59	0.65	0.002*	27
Erio25	$(CAA)_{10}$	EU410512	F: TTGTCACGAACATAAACGTA R: GTACATATTACAACAACAAC	100–106	50	3	0.93	0.58	0.000*	27
Erio29	$(GTT)_8$	EU410513	F: TACTCATCGCGAAAACGAGA R: AGTCTCGTCCGATGTTGTTG	171–189	60	6	0.67	0.71	0.000*	27
Erio33	$(CAA)_{12}$	EU410514	F: TCAATGGCAACCGAAGTGTA R: GCAACAGTGGCGTCATCC	159–183	60	9	0.78	0.85	0.210	27
Erio72	$(CT)_{13}$	EU410515	F: GCTGTAGCGGGCGTAATAAT R: AACCTTAACCGCCCCTCTAA	148–170	60	11	0.41	0.89	0.000*	27
Erio75	$(TC)_{12}(CT)_7$	EU410516	F: ACGGAGATGAAGGCGTTATG R: TCTCTCCGTCTTTCCGTCTC	134–166	60	7	0.48	0.73	0.000*	27
Erio78	$(AG)_{12}$	EU410517	F: AAGTTTAATGGCGTGGGCTA R: GGGATGGTAAACGAGTGTGTG	143–175	60	8	0.78	0.73	0.029*	27

37° south latitude) were used. PCRs were performed in a 10 μL reaction mixture containing: 1 ng/μL DNA template, 2.5 mM MgCl, 0.2 mM dNTP, 0.5 U *Taq* DNA polymerase (Invitrogen), 0.5 μM of each primer, 20 mM Tris-HCl, pH 8.4, 50 mM KCl. For all tested primers, thermal cycling conditions were carried out as follows: 3 min at 95 °C, followed by 35 cycles at 95 °C for 30 s, 30 s annealing (see Table 1 for annealing temperatures) and 1 min 30 s extension at 72 °C, with a final extension step at 72 °C for 5 min. Amplicons were separated in 6% polyacrylamide denaturing gels using a BIO-RAD Sequi-Gen GT Electrophoresis Cell. After electrophoresis, gels were silver-stained to visualize the PCR products using a procedure as described by Promega (1996). Variation at each locus was recorded by comparing the size of the amplicon in the gel (allele) in base pairs (bp) with the sequence of the PGEM 3ZF(+) vector (Promega Biosciences) loaded in the same gel. Hence, eight of the most polymorphic and reproducible primer sets are tested and characterized herein. Observed and expected heterozygosities were calculated using FSTAT (Goudet 2002), as well as linkage disequilibrium (LD) between loci. Significant deviations from Hardy–Weinberg equilibrium (HWE) were tested using the exact tests contained in Arlequin 3.11 (Excoffier *et al*. 2005). All microsatellite clone sequences were deposited in GenBank. Between three and 11 alleles per locus were observed for the 27 individuals (Table 1). While no LD was evident between loci, five loci (*Erio3*, *Erio20*, *Erio29*, *Erio72* and *Erio75*) revealed significant heterozygote deficiency and two loci (*Erio25* and *Erio78*) revealed significant homozygote deficiency. Analyses of loci using Micro-Checker 2.2.3 (Van Oosterhout *et al*. 2004) revealed the presence of null alleles for loci *Erio72* and *Erio75*, which could explain their significant heterozygote deficiency. Departures from HWE for other loci could be due to *E. lanigerum* in Chile reproducing predominantly through parthenogenesis. In combination with georeferenced data, these markers are currently being employed to determine the source of individuals infesting orchards during the apple production season, and to study the aphid–parasitoid association.

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