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PRIMER NOTE

Isolation of six microsatellite markers from the pea aphid, *Acyrthosiphon pisum* (Homoptera, Aphididae)

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Abstract

Using the magnetic particles method, we isolated six polymorphic microsatellite loci from the pea aphid, *Acyrthosiphon pisum* (Harris), which feeds on a wide range of legume species. The isolated loci were polymorphic, with three to six alleles in 40 aphids. Expected heterozygosities ranged from 0.12 to 0.65. These loci can be used to quantify clonal diversity and compare genetic population structure between sexual and asexual populations.

Keywords: Aphididae, asexuality, host race, magnetic particles, population genetics

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Acyrthosiphon pisum is a non host-alternating aphid that is associated with a wide range of legume species. This species is known as a complex of host races and subspecies with different host preferences (Blackman & Eastop 2000). Much attention has been concentrated on the mechanisms underlying host race formation (Via 1991; Hawthorne & Via 2001). Use of polymorphic microsatellite markers for this species facilitates the study of genetic population structure, genetic differentiation between populations, and the relationship between genetic diversity and selective pressures. Previous studies on *A. pisum* have used microsatellite markers that were originally developed for other aphid species (Caillaud *et al*. 2002; Wilson *et al*. 2004). We newly detected six polymorphic microsatellite loci for *A. pisum* for the purpose of comparing genetic population structure between sexual and asexual populations.

We constructed an enriched library based on the modified method of Fischer & Bachmann (1998). Genomic DNA was extracted from an aphid using the QIAamp DNA Mini Kit (QIAGEN). Five micrograms of genomic DNA was digested with 50 U of *Sau*3AI, and fragments were ligated to *Sau*3AI cassettes (TaKaRa). Polymerase chain reaction (PCR) amplification was carried out with Cassette Primer C1 (TaKaRa) in a PC-700 thermal cycler (ASTEC). The PCR products were resolved in binding buffer (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 7.5) and hybridized to 5′ biotin-labelled oligonucleotide probes $(CA)_{10}$, $(CT)_{10}$, and $(AAT)_7$ after denaturation. The hybrids were subsequently isolated by binding them to Streptavidin Magnetic Particles (Roche). After rinsing the particles in washing buffer (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 7.5), target DNAs were recovered by resuspending the particles in elution buffer (6 M Guanidine-HCl). The obtained fragments were then amplified by PCR and digested with *Sau*3AI to remove the cassettes. The enriched fragments ranging from 300 to 1000 bp in size were ligated into *Bam*HI-cut pUC118 (TaKaRa) and transformed into competent *Escherichia coli* cells (Competent High-DH5α, Toyobo).

A total of 1762 recombinant colonies were picked and were suspended independently in 20 μL of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). Inserts were amplified by PCR using a primer pair for the multicloning site of pUC18. After PCR, 1 μL of each amplified product was dropped separately on a positively charged MagnaGraph nylon membrane (Osmonics). After drying at room temperature, DNAs on the membrane were blotted by normal alkaline transfer. PCR products containing microsatellite regions were detected using a biotinylated DNA detection kit (Imaging High-Color, Toyobo) and 5′ biotin-labelled oligonucleotide probes mentioned above. Two hundred and eighty-seven of 1762 clones were positive. For positive clones, 1 μL of the remaining PCR product was checked for its length on 6% polyacrylamide gels in Tris-borate-EDTA. There are 81 unique inserts (length 300–1000 bp). Plasmids from the 81 positive clones were sequenced using an automated sequencer (CEQ 2000XL, Beckman Coulter). Of the 81 sequences, 20 primer pairs were designed using the online primer design software PRIMER 3.0 (Rozen & Skaletsky 1998).

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Table 1 Characteristics of the six microsatellites: repeat motif, number of alleles (N_a), size range in PCR product, primer sequences, locus-specific annealing temperature (T_a), observed (H_O) and expected (H_E) heterozygosities

Locus	Repeats	N_a	Size range (bp)	Primer sequences (5′–3′)	T_a (°C)	H_O	H_E	GenBank Accession no.
Ap-01	$(ATA)_5$	4	197, 201, 203, 209	F: AACCGGAGGGCCAAGACT R: ACGACGTCTAGGTCGAGCAC	58	0.48	0.65	AB162918
Ap-02	$(CG)_5$ … $(TTA)_7$	5	220, 222, 224, 229, 235	F: CGTCGCGACCTACCTGTAAT R: GGGTCCGGTGTAAAAATTGA	58	0.33	0.64	AB162919
Ap-03	$(GA)_{11}$	6	230, 232, 234, 236, 238, 240	F: GCAGCAAACAGCAGGTGTAAA R: ACAATTGCTCGATGGTCCTC	60	0.23	0.63	AB162920
Ap-04	$(AT)_9$	3	190, 192, 194	F: CCGCCATTTCGAGGCGGTAT R: GTCCGACATAATTGAGACGT	58	0.08	0.12	AB162921
Ap-05	$(AC)_7$ … $(AC)_5$	5	228, 240, 242, 248, 250	F: TCACCAAGGCATCCCTCTAC R: GTAGCGAATTTTTCCGGTTG	60	0.23	0.61	AB162922
Ap-06	$(TG)_8$	4	160, 170, 172, 174	F: CTGGGTTTATCCTCCGTGTG R: ACGGTTTCGGAGGCTAACTT	58	0.38	0.65	AB162923

To test effectiveness of the obtained microsatellite loci, we checked amplification and polymorphism of the primer pairs in 40 individuals of *A. pisum* collected from Chiba Prefecture and Hokkaido, Japan. PCR amplification was performed in 10-μL reaction volumes containing 1 μL of 10× reaction buffer, 0.8 μL of dNTP mix (2.5 mM of each dNTP), each 0.5 μL of primers (10 pmol/μL), 0.25 U of Gene *Taq* NT (Nippon Gene), 1 μL (50 ng) DNA and 6.15 μL of sterilized double-distilled water. Temperature cycles were as follows; 2 min at 94 °C; 30 cycles of 30 s at 94 °C; 30 s at 58–60 °C; 90 s at 72 °C.

Ten of 20 primers amplified the target regions successfully. Of these 10 loci, six showed polymorphism, with three to six alleles per locus (Table 1).

We tested for deviations of genotype frequencies from Hardy–Weinberg equilibrium for each locus in 40 aphids and linkage disequilibrium among the polymorphic loci using GENEPOP on the web (Raymond & Rousset 1996). A significant deviation was observed at four loci (Ap-02, Ap-03, Ap-05, and Ap-06, Hardy–Weinberg Exact Tests, $P < 0.001$), possibly reflecting inbreeding in *A. pisum* populations. There was no evidence for null alleles. Linkage disequilibrium was detected for three pairs of loci (Ap-01 and Ap-06, Ap-02 and Ap-06, Ap-05 and Ap-06, Linkage Disequilibrium, $P < 0.001$). We conclude that the markers are useful for assessing the extent of clonal diversity and for comparing genetic population structure between sexual and asexual populations.

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