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PRIMER NOTE

Microsatellite DNA markers for the pea aphid Acyrthosiphon pisum

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Abstract

Microsatellite loci were isolated from enriched partial genomic libraries of *Acyrthosiphon loti* and *Acyrthosiphon pisum*. Twenty of those loci were characterized in *A. pisum*. Fifteen of those loci were polymorphic. Genetic diversity varied across loci, allele repeat number ranging from two to 15, and observed heterozygosity from 0.1 and 0.96. An additional eight microsatellite loci originally isolated from other aphids but cross-priming with *A. pisum* showed polymorphism as well. Allele size ranged from three to 9 and observed heterozygosity from 0.43 to 0.84. Overall, we present 23 microsatellite loci that can be used to reveal polymorphism in pea aphids.

Keywords: cross priming, dinucleotide repeats, enriched library, microsatellite, pea aphid

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Aphids offer a unique advantage over many other arthropod systems for the study of the genetics of complex phenotypes because they are cyclically parthenogenetic. Every aphid hybrid lineage generated by sexual reproduction can be reproduced parthenogenetically, allowing replicate phenotypic measurements of each hybrid genotype. This replication is especially important for the study of genotype–environment interaction. The pea aphid, *Acyrthosiphon pisum* Harris (Homoptera, Sternorrhyncha) displays multiple advantages over other aphid species for development as a genetic system and is thus quickly becoming the workhorse genetic aphid system. However, few 'genetic' resources have been developed so far. In particular, only one linkage map, based on AFLP markers, has been published (Hawthorne & Via 2001).

Microsatellite markers harbour considerable length variation, are extremely abundant, and are randomly distributed throughout eukaryotic genomes (Jarne & Lagoda 1996). Consequently, they have found wide application as markers in human, mammalian, plant and invertebrate genetics, for the construction of highly informative and saturated genetic maps. Here, we characterize 23 microsatellite loci in the pea aphid. Fifteen of those loci are new. Eight of those loci have been previously characterized in three aphid species (Simon *et al*. 1999, 2001; Wilson *et al*. 2004) and we present here the result of cross-priming tests in the pea aphid.

Two separate microsatellite libraries were made. One with several genotypes of *Acyrthosiphon loti* L. (Homoptera, Sternorrhyncha) collected on *Lotus corniculata* L. plants in Montpellier (France) in 2002. One with a single genotype of *A. pisum* collected on *Medicago sativa* L. (alfalfa) in 1998 in Ithaca (NY state, USA).

Genomic DNA was isolated from fresh aphids following standard procedure (Sambrook *et al*. 1989) and digested with *Rsa*I restriction enzyme. A 300–1000 bp fraction of the digested DNA was selected on agarose gel, purified and ligated to Rsa linkers. The enrichment procedure followed the protocol from Kijas *et al*. (1994) based on streptavidin-coated magnetic particles (Magnesphere, Promega), with slight modifications. 5′-biotinylated $(CT)_{10}$ and $(GT)_{10}$ oligonucleotide were used as probes. The enriched single stranded DNA was amplified using one of the Rsa linkers as primer to recover double stranded DNA. The polymerase chain reaction (PCR) products were purified and ligated into pGEM-T Easy vector (Promega), and the plasmid transformed into *Escherichia coli* supercompetent cells (XL1 blue,

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Stratagene). Individual *E. coli* colonies were transferred to a positively charged Hybond-N + (Amersham) membrane. DNA was fixed on the membrane by baking at 80 °C for an hour. Positive colonies (i.e. containing indeed a microsatellite) were identified by hybridization with $(CT)_{10}$ and $(GT)_{10}$ probes labelled with digoxigenine using the DIG Nucleic Acid detection kit (Boehringer Manheim). 641 positive clones were picked and stored at −80 °C until sequencing.

Eighty-five of those positive clones were sequenced with an ABI 377 automated sequencer. We obtained 74 sequences, all containing repeated units. Pairs of primers were successfully designed for 61 of those sequences using the software PRIMER (version 2.0, Scientific & Educational Software 1990). PCR amplifications were attempted for 20 of those primer pairs.

PCR reactions were carried out in 8 µL of reaction mixture containing 0.75 µL 10X buffer, 0.9 µL 25 mm $MgCl_2$, 0.3 µL 10 mm dNTPs, 0.05 µL of Taq DNA polymerase 5 U/µL (Promega), 0.15 µL of 10 µmol forward and reverse primers and 1 µL of DNA (5 ng). Forward primers were labelled with IRD-800/700 fluorescent dye (LiCor) for scanner detection. Amplifications were performed on a PTC 225 MJ Research tetrad thermocycler. After an initial denaturing step of 2 min at 94 °C, 35 cycles were performed each consisting of 20 s at 94 °C, 20 s at the appropriate annealing temperature (56 °C or 60 °C) and 30 s at 72 °C. A final extension step was performed at 72 °C for 2 min.

PCR products were separated on 25 cm, 7.5% polyacrylamide gels (8.4 g urea, 3 mL LongRanger acrylamide (50%), qsp 20 mL 1X TBE, 225 µL APS (10%) and 22.5 µL Temed) run on a LiCor. A 65–385 bp Sizing Standard was run along with samples to determine allele sizes. The resulting products were scored using SAGA version 2.0 software.

Among the 20 loci tested, two were monomorphic and three showed unclear multiband patterns. Fifteen loci gave clear amplifications (Table 1) and were chosen for analysis of polymorphisms in *A. pisum* using a collection of 30 *A. pisum* genotypes collected in the same French location (Domagné,

Table 1 Characterization of 15 microsatellite loci in *Acyrthosiphon pisum*. Locus name, repeat in sequenced clone, primer sequences, locus-specific annealing temperature (T_a), size of the PCR product, number of observed alleles (30 genotypes tested), observed Heterozygosity (H_O), expected Heterozygosity (H_E). * isolated from *Acyrthosiphon loti*, † isolated from *Acyrthosiphon pisum*

Locus name	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Size range (bp)	# of alleles	H_O	H_E	GenBank accession number
AlA09M*	$(TC)_{12}$	F: CCTCTCACTCCATATCTCTC R: ACTTACAGTCCTCTGGCCAT	56	306–322	5	0.567	0.711	AY528722
AlA12M*	$(CT)_{12}$	F: ACCTACGACCAACGACACAC R: GATCACGAGACCTGCATTAC	60	414–438	7	0.667	0.766	AY528723
AlB04M*	$(GA)_{11}$	F: CAGCGCGCAGCGTATTATTA R: TGGTTCGTCGTGCTGTCTCG	56	242–252	6	0.828	0.725	AY528724
AlB07M*	$(AG)_{20}$	F: TACGGCGTGTCTCAGGTGCT R: ACAACTACCTAGGCCGACCA	60	117–159	15	0.967	0.864	AY528725
AlB08M*	$(CT)_{10}$	F: GCATGCTCGCACTCGCTTAG R: CGAAATACTGCCAAAACGGG	56	266–290	11	0.552	0.837	AY528726
AlB12M*	$(AG)_7AT(AG)_{18}$	F: GCTTAACGTCAGACGCTGAA R: GCCATAACAGAGACGTCATC	56	292–320	11	0.633	0.876	AY528727
AlC04M*	$(CA)_4T(AC)_4$	F: GCCTTCCCACAGAGCTATCG R: CTCGCTGTGTCCATCTTGAA	56	229–233	3	0.267	0.365	AY528728
AlC09M*	$(GT)_6$	F: CGACAGTTAGCGTGCATGTT R: ATCGTCACCACTACCGTCGT	56	210–218	2	0.267	0.231	AY528729
ApF08M†	$(TG)_2TA(TG)_8$	F: TAATCCGTCGTAATTGCGTT R: TAAGCCCTCACTCACCCCTC	56	163–174	6	0.600	0.625	AY528730
ApG10M†	$(GCT)_8$	F: CAACGACGGCGGCTATACTA R: ACGAGAGCTTTCCGGCGTAT	56	175–178	2	0.133	0.231	AY528731
ApH 04M†	$(AC)_5GAAT(AC)_4$	F: CGCATCGAGTGTCGTATTAT R: GTTCCAAGGTCCTCTCTTCC	56	256–260	2	0.103	0.098	AY528732
ApH 05M†	$(AGC)_8$	F: ACGAGAGCTTTCCGGCGTAT R: CAACGACGGCGGCTATACTA	56	174–182	3	0.167	0.283	AY528733
ApH 08M†	$(CA)_{10}$	F: GCGCACAGTGCGTATACATT R: TATTACAACGCACGTCATCG	56	250–284	10	0.833	0.757	AY528734
ApH 10M†	$(CA)_{16}$	F: ACGACGGGTGCAAGTATATT R: CAACATGACCTCGCTTCAGA	56	186–208	8	0.567	0.636	AY528735
ApH 12M†	$(CA)_7$ … $(TA)_3T(CA)_3$	F: CTTCCACAAGAAACTCCGGT R: CTCGGTAACCACCTTGGTAG	56	225–228	2	0.172	0.158	AY528736

Table 2 Characterization of eight microsatellite loci isolated from Sitobion and Rhopalosiphum species in Acyrthosiphon pisum. Locus name, repeat in sequenced clone, primer sequences, locus-specific annealing temperature (T_a), size of the PCR product, number of observed alleles (30 genotypes tested), observed Heterozygosity (H_O), expected Heterozygosity (H_E), reference. * isolated from Sitobion miscanthi, † isolated from Sitobion avenae, ‡ isolated from *Rhopalosiphum padi*

Locus name	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Size range (bp)	# of alleles	H_O	H_E	References
Sm11*	$(AC)_9$ $(AC)_5$	F: GGTACCCCTATGTTATTACGCG R: AACCCTACGGGTAACGCC	56	125–151	8	0.533	0.752	Simon *et al*. (1999)
S23*	$(CT)_{14}$	F: GGTCCGAGAGCATTCATTAGG R: CGTCGTTGTCATTGTCGTCG	56	126–142	8	0.633	0.754	Wilson *et al*. (2004)
S17b*	$(CA)_{11}TA(CA)_8(TA)_7$	F: TTCTGGCTTCATTCCGGTCG R: CGTCGCGTTAGTGAACCGTG	56	216–222	4	0.633	0.682	Wilson *et al*. (2004)
S30*	$(CA)_{13}$	F: CCGACATAAAACACACCCAG R: GTTTTGCCTCCTCCCCTC	60	162–172	6	0.846	0.808	Wilson *et al*. (2004)
S3.43†	mc $(ATT)_7$.mc $(TG)_{10}CG(TG)_6$.mc	F: GGCGAGACCCCTTAAAATCC R: GAGATACTCTTTTCGTTAAACC	56	165–189	9	0.600	0.758	Wilson *et al*. (2004)
S5.L†	mc$(TG)_{10}$mc	F: GGACGACTCGTTAGTATAGGTGG R: CTATCTCTACCGTTTCGAATCG	56	204–207	3	0.433	0.611	Wilson *et al*. (2004)
R5.29‡	$(AC)_{14}$	F: GTTTTAATTTCCTCCACGC R: GTTAAAAGGGACACACTCATG	56	171–181	6	0.767	0.764	Simon *et al*. (2001)
R5.10‡	$(CA)_3AC(AG)_6(ATT)_5$ $(GA)_{15}$	F: CCGACTAAGCTTAATATTGTTTG R: CGGTTCGGAGAACATAAGAG	56	230–276	8	0.800	0.745	Simon *et al*. (2001)

Brittany) but from different host plants (i.e. 10 from pea, clover and alfalfa, respectively). Allele number varied across loci and ranged from two to 15 alleles per locus. The averaged observed heterozygosity (H_O) ranged from 0.1 to 0.96, and the expected heterozygosity (H_E) ranged from 0.09 to 0.87.

Polymorphism in pea aphids was also studied for eight microsatellite loci isolated from three other aphid species: *Sitobion avenae* F. (Wilson *et al*. 2004), *S. miscanthi* L. (Simon *et al*. 1999; Wilson *et al*. 2004), and *Rhopalosiphum padi* L. (Simon *et al*. 2001). Variation in allele number ranged from three to 9 alleles per locus, the averaged observed heterozygosity (H_O) ranging from 0.43 to 0.84 and the expected heterozygosity (H_E) ranging from 0.61 to 0.8 (Table 2). Interestingly, the level of polymorphism observed with the cross priming was as high as the one observed with primer pairs designed specifically for the species *A. pisum*. None of the 23 microsatellite loci examined on the French sample of *A. pisum* showed highly significant (i.e. $P < 0.01$) deviation from Hardy–Weinberg proportions nor linkage disequilibrium when tested with the GENEPOP software (Raymond & Rousset 1995). The 23 microsatellite loci described in this study will be of immediate use for population genetics studies of pea aphids. Our long-term goal is to characterize enough microsatellite loci to generate a dense linkage map for *A. pisum* for fine-scale mapping map-based cloning approaches. If we extrapolate the results obtained with the two libraries constructed from *A. pisum* and *A. loti*, we can expect the 641 positive clones identified to yield 558 useful sequences, 459 primer pairs, and 344 polymorphic microsatellite loci.

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