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PRIMER NOTE

Isolation of microsatellite markers from the drepanosiphid aphid *Tuberculatus quercicola* (Homoptera, Aphididae)

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Abstract

We isolated five polymorphic microsatellite loci from the drepanosiphid aphid *Tuberculatus quercicola* (Matsumura) that is associated with the Daimyo oak, *Quercus dentata* Thunberg, using the magnetic particles method. The isolated loci were polymorphic, with four to 16 alleles in 40 aphids. Expected heterozygosities ranged from 0.4 to 0.82. These loci can be used to quantify seasonal changes in clonal diversity in the metapopulation and the extent of clonal mixing in the colonies.

Keywords: Aphididae, magnetic particles, myrmecophillous, population genetics, *Quercus dentata*

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The aphid *Tuberculatus quercicola*, a non host-alternating species, is associated with the Daimyo oak, *Quercus dentata*, on which the colonies are always attended by some ant species. In May the nutritional quality of host leaves is so high that a high reproductive rate is realized in *T. quercicola* colonies. However, with the advance of seasons, a significant reduction is found in both the total amino acid concentration in phloem sap and the frequency of honeydew excretion (Yao & Akimoto 2002). To keep food quality high and maintain ant attendance, it is expected that aphids migrate to favourable feeding sites in a host plant and form new colonies consisting of mixed clones as the seasons progress. To investigate the extent of clonal mixing we developed five microsatellite markers in *T. quercicola*.

We constructed an enriched library based on the modified method of Fischer & Bachmann (1998). Genomic DNA was extracted from 50 aphids using the QIAamp DNA Mini Kit (QIAGEN). Five μg of genomic DNA were 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 oligonucleotid probes $(CA)_{10}$ 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 750 recombinant colonies were picked and were suspended independently in 20 μL of TE (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 oligonucleotid probes $(CA)_{10}$. A hundred and twenty-two of 750 clones were positive. For positive clones, 1 μL of the remaining PCR product was checked for its length on 6% polyacrylamide gels in TBE. There are 43 unique inserts (length 300–1000 bp). Plasmids from the 43 positive clones were sequenced using an automated sequencer (CEQ 2000XL, Beckman Coulter). Of the 43 sequences, 21 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 five microsatellites: repeat motif, size range in PCR product, number of alleles, primer sequences, locus-specific annealing temperature, observed and expected heterozygosities

Locus	Repeats	Size range (bp)	No. of alleles	Primer sequences (5′–3′)	T_a (°C)	H_O	H_E	GenBank No
Tq-15	$(GT)_{21}$	220–290	16	CAACGGTGAGGAGGATGTTT ACGCCACCACTACGTTCTTC	55	0.60	0.82	AB106557
Tq-17	$(GT)_{11}$	229–237	4	GTATGCGTGTGTCGAAATGC TTCCGACCCATCGATTCTAC	57	0.23	0.4	AB106558
Tq-18	$(GT)_{13}$	112–118	4	ACAATACAGTGTGCGTGTGC CCGGCAATTATGAGAGCATC	57	0.13	0.48	AB106559
Tq-23	$(GT)_{12}$	172–190	7	CCTGTACGGCGTTTATTGCT GCGCGTGAAACATTTTTGTA	55	0.10	0.76	AB106560
Tq-26	$(GT)_{15}AT(GT)_9$	170–177	6	AGTGCGCGGGTCTTATATGT AACTCACGGTTCTGCGTGTA	53	0.08	0.76	AB106561

To test effectiveness of the obtained microsatellite loci, we checked amplification and polymorphism of the primer pairs in 40 individuals of *T. quercicola* collected from our study sites, Hokkaido, northern Japan. After optimization of PCR conditions, forward primers were labelled with Beckman Dye fluorescence (Proligo). PCR amplification was performed in 10 μL reaction volumes containing 1 μL of 10Xreaction buffer, 0.8 μL of dNTP mix (2.5 mM of each dNTP), each 0.25–0.5 μL of primers (10 pmol/μL), 0.25 U of Gene *Taq* (NIPPON GENE), 1 μL (50 ng) DNA and 6.15–6.65 μL of sterilized ddW. Temperature cycles were as follows; 2 min at 94 °C; 30 cycles of 20 s at 94 °C; 15 s at 53–55 °C; 1 min at 72 °C. The genotype was determined using an automated sequencer mentioned above.

Eleven of 21 primers amplified the target regions successfully. Of these 11 loci, five showed polymorphism, with four to 16 alleles per locus (Table 1). Observed heterozygosities were lower than expected heterozygosities at all loci; possibly reflecting inbreeding in *T. quercicola* populations. We conclude that the markers are useful for assessing the extent of clonal mixing in *T. quercicola* populations.

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