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PRIMER NOTE

# Isolation and characterization of microsatellite loci in the aphid species, *Rhopalosiphum padi*

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**Abstract**

**Due to their properties for resolving patterns of population genetic structure, microsatellites are increasingly used in studies of breeding systems. Here, eight polymorphic microsatellite loci were isolated and characterized in the aphid *Rhopalosiphum padi*, in which populations show a mixture of cyclically and obligatory parthenogenetic lines. These loci were then applied to distinguish between 55 parthenogenetic lines of *R. padi* collected across France. Interestingly, they allowed to detect several copies of the same genotypes among the sample, confirming the great sensitivity of microsatellites and their usefulness in population genetic studies of parthenogenetic organisms.**

*Keywords*: aphid, breeding system, microsatellite markers, genetic diversity, *Rhopalosiphum padi*

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Breeding system studies (e.g. evolution of sex and selfing rates) have considerably benefited from the recent advance of microsatellite markers. First, microsatellites extend the range of organisms suitable for studies on breeding system diversity that previously showed little genetic variation (e.g. Jarne & Lagoda 1996). Second, they are powerful tools to infer the mode of reproduction of a species from detailed analysis of its population genetic structure (e.g. Awadalla & Ritland 1997). Third, they can be very useful for understanding the evolutionary biology of breeding systems at the infraspecific level (e.g. Simon *et al*. 1999).

In this note, we report on the isolation of eight polymorphic microsatellite markers in the aphid *Rhopalosiphum padi* L. (Hemiptera, Aphididae). This species, which commonly feeds on cereals, shows two main lineages which reproduce by cyclical parthenogenesis (i.e. several parthenogenetic generations followed by a single annual sexual generation) and by obligate parthenogenesis (i.e. apomictic clonal reproduction), respectively (Simon *et al*. 1996). This breeding system polymorphism renders this organism a useful model for understanding the ecological and evolutionary basis for maintenance of sex. However, these studies have been constraint by the lack of highly polymorphic markers (Simon *et al*. 1996; Hales *et al*. 1997).

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Microsatellite isolation followed the procedure of Estoup & Turgeon (1996). Briefly, DNA was extracted from 100 aphids using the salting-out method (Sunnucks *et al*. 1996) and a partial library was constructed after complete digestion of DNA by *Sau*3AI. Fragments of 500–1000 bp were ligated into a pUC18 plasmid (Pharmacia Biotech) and transformed using XL1-blue competent cells (Stratagene). A set of digoxigenine-labelled probes [$(TG)_{10}$, $(CAC)_5CA$, $(TGTA)_6TG$] was used to screen 1200 recombinant colonies and inserts from 20 positive clones were sequenced. Eleven loci containing microsatellite arrays were then selected for primer design using the PRIMER3 program (Rozen & Skaletsky 1997).

Polymorphism was assessed on a collection of 55 parthenogenetic lines of *R. padi* sampled mainly across northern France and maintained alive in the laboratory. DNA was extracted from single individuals of each aphid line using the salting-out method (Sunnucks *et al*. 1996). Microsatellite loci were amplified in a final volume of 15 μL using 3 pmol of each primer, 50 μM of each dNTP, 1.5 μL of 10× $Mg^{++}$ free reaction buffer (500 mM KCl, 100 mM Tris-HCl pH 9.0 and 1.0% Triton X-100), 2 mM of $MgCl_2$, 0.5 U of *Taq* DNA polymerase (Promega) and 1 μL of DNA (5 ng).

**Table 1** Characteristics of the eight microsatellite loci isolated from *Rhopalosiphum padi*: locus name, core repeat in sequenced clone, primer sequences, locus-specific annealing temperature ($T_a$), number and size range in base pair of alleles at each microsatellite locus, observed ($H_O$) and expected ($H_E$, i.e. 'gene diversity', Nei 1987) heterozygosities and accession numbers in GenBank

| Locus | Repeat motif in library | Primer sequences (5′–3′) | $T_a$ (°C) | No. of alleles | Size range (bp) | $H_O$ | $H_E$ | Accession numbers |
|---|---|---|---|---|---|---|---|---|
| R1.35 | $(TC)_{13}$ | F: CGCCGCATAGCCTCCC<br>R: CTCGTTATTGCGGTATTGCTTTG | 60 | 10 | 345–360 | 0.62 | 0.83 | AF277461 |
| R5.10 | $(CA)_3AC(AG)_6(ATT)_5\ldots(GA)_{15}$ | F: CCGACTAAGCTTAATATTGTTTG<br>R: CGGTTCGGAGAACATAAGAG | 60 | 8 | 256–274 | 0.82 | 0.79 | AF277462 |
| R2.73 | $(GT)_{25}GG(ATT)_4$ | F: CGTAGACCGCCGCGGG<br>R: GTCGTTTCTGGTCAGCGGCC | 60 | 6 | 262–285 | 0.87 | 0.78 | AF277466 |
| R5.29.b | $(AC)_{13}$ | F: CATGAGTGTGTCCCTTTTAAC<br>R: GATGGACGAGGGGACAC | 60 | 17 | 161–216 | 0.87 | 0.81 | AF277464 |
| R6.3 | $(AT)_7A(AT)_6(TA)_3$ | F: CGAAATGTACCCACTATAAAC<br>R: CAAATTTAAATGTATAATCAATG | 52 | 5 | 161–183 | 0.35 | 0.3 | AF277465 |
| R3.171 | $(AT)_2T(AT)_9\ldots(AC)_{11}$ | F: TGTACATCGTAAGACGTAAAACGAC<br>R: CAAAGCAATACCGCATAACG | 60 | 15 | 214–252 | 0.46 | 0.81 | AF277463 |
| R5.138 | $(CA)_{35}$ | F: TATACACGCTCGCGCTTACG<br>R: CCGAGCACGAATTGTTCC | 60 | 20 | 211–287 | 0.81 | 0.83 | AF349565 |
| R5.50 | $(AC)_{22}$ | F: TGTTACGCGGAGTGTGTAGG<br>R: CCACAGAGCGTTGTCATC | 60 | 25 | 297–403 | 0.84 | 0.87 | AF349550 |

Polymerase chain reactions (PCR) were performed on an Hybaid thermocycler with the following programme. One denaturation step at 94 °C for 2 min followed by 35 cycles with a denaturation step at 94 °C for 20 s, 20 s at a locus-specific annealing temperature (Table 1) and an elongation step at 72 °C for 20 s; then a final cycle at 72 °C for 2 min. Amplification products were resolved on a 6% polyacrylamide urea electrophoresis gel and visualized after nitrate silver staining as described by Budowle *et al*. (1991). Allele sizes were assigned using the sequence of the pGEM®-3Zf(+) vector (Promega).

Of the 11 loci tested, eight were polymorphic, one was monomorphic and two were not suitable as they produced dubious amplification patterns. Polymorphic loci had between five and 25 alleles and expected heterozogosities ranged between 0.30 and 0.87 (Table 1). Forty different eight-locus genotypes (clones) were detected among the 55 parthenogenetic lines of *R. padi*. In particular, three genotypes were each found up to six times in the sample. This result emphasizes the great sensitivity of microsatellites for detecting clonal copies in parthenogenetic organisms, as previously illustrated by Sunnucks *et al*. (1997) on aphids or Gomez & Carvalho (2000) on rotifers.

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