# Microsatellites from *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae), a specialist primary parasitoid attacking the specialist tansy aphid, *Metopeurum fuscoviride* Stroyan (Hemiptera: Aphididae)

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**Abstract**

**Nine polymorphic microsatellite loci were isolated from the specialist aphid parasitoid, *Lysiphlebus hirticornis*. In addition, two published loci from closely related *Lysiphlebus* species were also used. Allelic diversity and heterozygosity were quantified in samples collected from eight tansy plants growing in an area of approximately 150 m² in Jena, Germany.**

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The aphid, *Metopeurum fuscoviride* Stroyan, a specialist on tansy (*Tanacetum vulgare* L.), shows a metapopulation structure with colonization and extinction phases on its plant host (Weisser 2000; see also Massonnet *et al*. 2002). It is attacked by two specialist braconid parasitoids, *Aphidius tanacetarius* Mackauer and *Lysiphlebus hirticornis* Mackauer (Starý 1973). The population structure and dynamics of the aphid host may influence the population genetic structure and dynamics of the specialist parasitoids (Weisser 2000; Rauch & Weisser 2007). Here we investigate the population genetic structure of *L. hirticornis* in the light of the behaviour and ecology of *M. fuscoviride*, whose distribution reflects that of tansy, in effect a tri-trophic study.

Genomic DNA was isolated from a pool of 40 female wasps following Sunnucks & Hales (1996), resuspended in 100 μL TE buffer, pH 8.0, and stored at −20 °C. Cloning procedure followed Jones *et al*. (2002). Purified DNA was partially restricted using a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *BsrB*1, *Pvu*II, *Stu*I, *Sca*I, *Eco*RV). Fragments between 350 bp and 700 bp were isolated and ligated to adaptors containing a *Hin*dIII site. Magnetic bead capture (CPG Inc.) was used following the manufacturer's protocol to isolate microsatellite-containing fragments, using biotin-$(CA)_{15}$, biotin-$(GA)_{15}$, biotin-$(ATG)_{12}$ and biotin-$(TAGA)_8$ as capture molecules. These were amplified and digested with *Hin*dIII to remove the adaptors, and ligated into the *Hin*dIII site of pUC19 vectors. Recombinant molecules were electroporated into *Escherichia coli* DH5α (ElectroMaxJ, Invitrogen); a 2:1 ligation mix was used for each of the genomic libraries. After transformation and recovery incubation in SOC broth (Invitrogen), glycerol was added (20% final vol.) and the products plated out to produce 100–300 recombinant colonies. Colonies were isolated for sequencing on bluo-gal-IPTG/ampicillin LB (BIA-LB) plates, and incubated at 37 °C using 2× LB broth in a 96-well block. Plasmid DNA was purified from randomly selected recombinant clones using Millipore MultiScreen MAFB NOB plates (http://www.millipore.com/), and sequencing was performed using DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences) on an Applied Biosystems 377 Sequencer. Enrichment levels were expressed as the fraction of sequences containing a microsatellite, from which 23 polymerase chain reaction (PCR) primers were designed using DesignerPCR software version 1.03 (Research Genetics, Inc.).

An initial screening for polymorphism in the 23 primers with seven female parasitoid samples on 1.5% agarose gel yielded six polymorphic loci. Forward primers for the six were fluorescently labelled (LI-COR IRD 700 and 800) and routine survey begun. One primer was, however, discovered to have a duplicated primer-binding site and was thus discarded. In order to screen for more polymorphic loci, M13 adapter tails were made for the remaining 17 primers together with 11 loci developed from *Lysiphlebus fabarum* (Sandrock *et al*. 2007) and six from *Lysiphlebus testaceipes*

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