Molecular Ecology Notes (2004) **4**, 605–607 doi: 10.1111/j.1471-8286.2004.00751.x

PRIMER NOTE

Microsatellite isolation from the gall midge *Spurgia capitigena* (Diptera: Cecidomyiidae), a biological control agent of leafy spurge

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Abstract

Spurgia capitigena **is a gall midge that has been released as a biological control agent of leafy spurge (*Euphorbia esula*), a noxious rangeland weed in North America. We isolated 15 microsatellite loci from *S. capitigena* with from two to 27 alleles at each locus. These markers will allow us to examine the structure and levels of neutral genetic variation in native and introduced populations.**

Keywords: biological control, gall midge, genetic diversity, leafy spurge, microsatellites

Received 9 April 2004; revision accepted 23 June 2004

Biological control has been used to regulate pest populations for hundreds of years; however, few studies have focused on the genetic diversity of biological control agents. Due largely to logistical constraints, most biological control agents are collected as small subpopulations that may not represent the full genetic diversity of the source population. This reduced genetic diversity among the natural enemies could modify their effectiveness (Hopper *et al*. 1993). The leafy spurge gall midge (*Spurgia capitigena*) was introduced into North America in 1985 as a biological control agent of leafy spurge (*Euphorbia esula*). Given the potential utility of biological control agents, it is particularly critical to understand the effects of biological control introductions on both neutral and ecologically important traits. To this end, we developed microsatellite markers for *S. capitigena*.

Genomic DNA from *S. capitagena* was obtained from an introduced population in Kindred, North Dakota for microsatellites SC1, SC2, SC3, SC5, GTT6, AC15 and TG11, and from a mixture of DNA extractions from different Italian and French fly populations for the remaining microsatellites. Midges were killed by freezing at –80 °C, and their genomic DNA was extracted using the DNeasy™ Tissue Kit (Qiagen) with the following modifications. A total of 165 midges (0.079 g) were used as starting material for the first set of loci. For this extraction, the first four steps of the protocol for animal tissues (DNeasy™ Tissue Kit Handbook, April 1999, Qiagen) were scaled up 1.5 times. DNA was eluted with 180 µL buffer AE after incubating for 5 min at room temperature. For the second set of loci, genomic DNA from about 20 individual wasps was pooled.

Microsatellite cloning and sequencing was performed at the Evolutionary Genetics Core Facility of Cornell University following a modification of the protocol of Hamilton *et al*. (1999). The genomic DNA was digested with *Alu*I and *Hae*III, an SNX linker was ligated, and the DNA was enriched for repeats by hybridization to a mixture of repetitive biotinylated di-, tri- and tetra- oligonucleotides (all possible combinations beginning with G or T and excluding the reverse, compliments and homopolymers) and magnetic capture with streptavidin-coated magnetic beads. Enriched fragments were made double-stranded by polymerase chain reaction (PCR), digested with *Nhe*I, and cloned into *Xba*I-digested, dephosphorylated pUC 19. Colonies were grown on Luria-Bertani plates with ampicillin, and replicas were transferred to nylon membranes and probed with radiolabelled oligonucleotides. Positive colonies were sequenced with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and a universal M13 primer that flanks the cloning site. Reactions cycled at

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Table 1 Characteristics of 15 microsatellite loci in *Spurgia capitigena*: locus name, GenBank Accession no., repeat structure, primer sequences, size of sequenced allele, PCR annealing temperatures (T_a), number of alleles, size range of amplified alleles, average observed (H_O) and expected (H_E) heterozygosities

Locus (Accession no.)	Repeat	Primer sequence (5′–3′)	Size (bp)	T_a (°C)	Number of alleles	Size range (bp)	H_O	H_E
SC1 (AY568935)	$(TG)_8$	F: FAM-TACATTCCGCTCACATACCTCATACCAT R: CGTTTACACTGATGCGTGAATAATAGCA	284	57	9	250–304	0.14	0.22
SC2 (AY568936)	$(TG)_{12}$	F: NED-TGTAAAACAGCAAACGGCACATAGAG R: AATCGTCTTCTGTTTTGCATTTA	170	61	2	168–170	0.12	0.38
SC3 (AY568937)	$(AC)_{14}$	F: FAM-TGATGGTTTGCAATATTTTTCGTA R: TGCGATCATTCCAAACCATT	181	61	10	163–189	0.21	0.59
SC5 (AY568938)	$(CT)_{20}$	F: HEX-GCTTCAGGCACTTTAATGGTATGTTGTT R: AGAGCAATAAAATAAAACGGCAAACGAT	160	57	16	150–186	0.53	0.81
GTT6 (AY568939)	$(GTT)_6$	F: HEX-GCAGAAGCACTTCCTCATAC R: CGAAAACCGCCATACTATAC	191	50	2	190–193	0.00	0.02
AC15 (AY568940)	$(AC)_{15}$	F: HEX-TGTAATGTCTGTTTGTGGTTTGTC R: TCTTAGTTGATGGTTCATTTGGAC	158	62	11	139–189	0.46	0.54
TG11 (AY568941)	$(TG)_{11}$	F: HEX-ATTATTATAGCCATCGATGTCAAG R: GCGTGTTCATAGCTTCCTTCTC	171	60	13	157–203	0.54	0.66
SC52 (AY568942)	$(GT)_{61}$	F: HEX-TATTTTCTCTGCTCTCTTAC R: TGATACACATTCCGTTTG	321	54	22	303–347	0.60	0.89
SC57 (AY568943)	$(CA)_8$	F: NED-CAGCCACAATGTGAATAC R: CTGGATCTACAAATGAGTG	146	58	11	139–189	0.54	0.68
SC65 (AY568944)	$(GTT)_9$	F: NED-AACGAACAGTTCCAATGATTC R: TTCTGCCAACGAAGCCAC	139	64	3	119–137	0.26	0.53
SC68 (AY568945)	$(GT)_{15}$	F: NED-CGAGGAGATGGATGAATG R: TCAGACGCGTAGAATAACTG	396	54	10	376–412	0.16	0.59
SC70 (AY568946)	$(GTT)_7$	F: HEX-GAAACAGTAATCGCAAAC R: GTAATGAGACAAAGGTAGC	258	59	2	257–260	0.17	0.17
SC75 (AY568947)	$(CT)_{22}$	F: FAM-TTTGCTCTTCTCTCTCTC R: TCCATATATTCGTCATTG	148	53	27	126–194	0.61	0.87
SC77 (AY568948)	$(GT)_{24}$	F: FAM-TGCTAACTATATTTCCGAACA R: GGCATGCCCTTTCAACAC	118	56	19	97–157	0.32	0.81
SC81 (AY568949)	$(AACC)_4$	F: FAM-GCCCATAGCCTTCAATAAAG R: TTCGATCGACGATGTGTTC	373	63	2	370–374	0.08	0.13

95 °C for 50 s, 50 °C for 20 s, 60 °C for 4 min (25 cycles total) then were analysed on an ABI 377 Automated Sequencer.

Primers were designed using Primer Select (DNAStar). Forward primers were labelled using the fluorescent dyes 6-FAM™ (Integrated DNA Technologies), HEX™ (Applied Biosystems) and NED™ (Applied Biosystems). Microsatellite loci were amplified using PCR in 10 µL volumes consisting of: 1 × PCR Buffer (20 mM Tris-HCL, pH 8.4, 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U *Taq* polymerase (Novagen), 0.1 µL *Taq*Start antibody (Clontech), 2 pmol of each primer, and 1 µL of extracted genomic DNA. The amplification cycles consisted of 95 °C for 60 s for initial denaturation followed by 35 cycles of: 95 °C for 50 s, the annealing temperature (see Table 1) for 60 s, and 72 °C for 90 s. A final extension step of 10 min at 72 °C was used at the end of the program. PCR products were held at –20 °C before genotyping. In this initial test for developing these primers, we report pooled results on 174 *S. capitigena* from France, Italy and the US.

Genotyping was performed on an ABI 3100 capillary Genetic Analyser at Iowa State University's DNA Sequencing and Synthesis Facility. A reference DNA sample was sent out with each order to ensure consistency in scoring. Results were sent electronically and all scoring was done with the GenoGrapher AFLP software (http://hordeum.oscs.montana.edu/genographer/).

Observed (H_O) and expected heterozygosities (H_E) and linkage disequilibrium at each locus were calculated using GENEPOP on the web (http://wbiomed.curtin.edu.au/genepop; Raymond & Rousset 1995). Observed heterozygosities were typically much lower than expected at most loci (Table 1). The number of alleles per locus ranged from 2 to 27 (Table 1), which may be a consequence of the sib-mating that is thought to be typical of these short-lived flies. Individuals sampled for these analyses came from several locations, thus we tested for linkage disequilibrium within sample locations. No linkage disequilibrium was detected in six of the eight locations,

suggesting that the loci presented here are not physically linked. These microsatellite markers should prove valuable markers for exploring the population genetics of this species.

Acknowledgements

We thank Rouhollah Sobhian, Gaetano Campobasso, Gianni Terragitti and Boris Fumanal at the European Biological Control Laboratory, Denise Olson at North Dakota State University, Raul Ruiz at the USDA APHIS PPQ and Don Mundal for providing us with samples. This research was funded by the USDA-NRI Project Award no. 2001-35320-10020 to SJN.

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