Molecular Ecology Notes (2002), **2**, 346–348 doi:10.1046/j.1471-8278 .2002.00246.x

PRIMER NOTE

Characterization of microsatellite loci in the parasitoid, *Cotesia congregata* (Say) (Hymenoptera Braconidae)

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Abstract

Nine microsatellite loci were developed and assayed for polymorphism in four groups of the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera). One locus was monomorphic and eight were polymorphic in *C. congregata*. Allelic distributions differed significantly between groups of *C. congregata* collected from solanaceous and catalpa host food plants ($P = 0$). Loci were tested for cross-reactivity to six congeners: *C. acuminata* (Reinhard), *C. bignellii* (Marshall), *C. cynthiae* (Nixon), *C. euphydryidis* (Musebeck), *C. koebelei* (Riley) and *C. melitaearum* (Wilkinson). In the congeners, eight loci were polymorphic, four of which yielded allele sizes that differed substantially from sizes of amplification products observed for *C. congregata*.

Keywords: *Cotesia*, local adaptation, tritrophic interactions

Received 22 January 2002; revision received 25 April 2002; accepted 25 April 2002

Cotesia congregata (Say) is an endoparasitoid of larval sphingids (Krombein *et al*. 1979). Most of its hosts are specialists on one or a few plant families (Hodges 1971), many of which are noxious. A tritrophic interaction exists between *C. congregata,* its hosts and the plants on which these hosts feed, and field-based evidence suggests that this parasitoid may be locally adapted to host food plants (Kester & Barbosa 1991, 1994). Two common hosts of *C. congregata*, *Manduca sexta* L. and *M. quinquemaculata* (Haworth), are specialists on solanaceous plants, including tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*). Another host, *Ceratomia catalpae* (Boisduval), feeds exclusively on catalpa (Bignoniaceae: *Catalpa* spp.).

Detection of microsatellite sequences in a size-selected [400–900 base pairs (bp)] partial genomic library (pBluescript II SK+) derived from the pooled DNA of six *C. congregata* wasps was performed as described by Rassmann *et al*. (1991). Transformant colonies were screened using a cocktail of digoxygenin-labelled oligonucleotide probes [$AG(CAG)_6CA$, $(ATT)_{12}$, $TG(CTG)_6CT$, $(GAC)_6$, $(AAG)_9$, $(GTA)_7$ and $(ATG)_7$]. Polymerase chain reactions contained 100 ng genomic DNA, 1.0 μM each primer, 0.2 mM each dNTP, 0.45 units of *Taq* polymerase (Invitrogen), 20 mM Tris–HCl, pH 8.4, 1.5 mM $MgCl_2$, 50 mM KCl, and sufficient distilled H_2O for a total volume of 15 μL. Amplification was conducted in PTC-100 thermal cyclers (MJ Research) using an initial denaturation at 94 °C for 1 min, followed by 38 cycles of 94 °C for 30 s, 55–65 °C for 30 s, and 72 °C for 15 s. Amplification products were resolved by polyacrylamide gel electrophoresis. Mendelian inheritance of alleles was determined by examining the amplified products in two or more families per locus (parent and 10 offspring per family). Polymorphism was assayed in four groups of *C. congregata* collected within Virginia from *M. sexta* or *M. quinquemaculata* on tomato or tobacco ('solanaceous group') in Blackstone (latitude 37°05′ N, longitude 077°59′ W; $n = 64$) and Richmond (latitude 37°32′ N, longitude 077°27′ W; $n = 12$), and from *C. catalpae* on catalpa ('catalpa group') in Charlottesville (latitude 38°02′ N, longitude 078°31′ W; $n = 8$) and Richmond (latitude 37°32′ N, longitude 077°27′ W; $n = 12$). Primers were also tested with six congeners: *C. acuminata* (Reinhard) ($n = 38$), *C. bignellii* (Marshall) ($n = 21$), *C. cynthiae* (Nixon) ($n = 5$), *C. euphydryidis* (Musebeck) ($n = 5$), *C. koebelei* (Riley) ($n = 5$) and *C. melitaearum* (Wilkinson) ($n = 193$), found on a variety of hosts. Statistical analyses were performed using GENEPOP Ver 3.1 (Raymond & Rousset 1995). Because of the haplodiploid nature of inheritance, only female data were included in analyses of observed and expected heterozygosity.

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Table 1 Attributes of eight polymorphic microsatellite loci examined in *Cotesia congregata* (Say) from four native groups collected from *Manduca sexta* L. or *Manduca quinquemaculata* (Haworth) on tobacco or tomato in Blackstone and Richmond, VA (n = 76), and from *Ceratomia catalpae* (Boisdivul) on catalpa in Charlottesville and Richmond, VA (n = 20), the latter group shown in parentheses

Locus Repeat	GenBank accession number	Primer sequences (5′–3′) (F = forward, R = reverse)	T_a (°C)	SR (bp)	A	H_O	H_E	HWE
Cco-1A	AF453312	F: TCAGTTCCATATGCTGCA	55	140–158	5	0.10	0.28	0*
$(CTG)_8(TTG)_{16}$		R: TGATGCGTCATTCCTTGA		(137–164)	(3)	(0.13)	(0.13)	—
Cco-5A	AF453313	F: ATGCAAGGCGCCACGATTT	60	89–116	5	0.18	0.32	0*
$(TCA)_{10}$		R: AATTTACGAGTCGATTAGTATGTTAATACGGAT		(101–104)	(3)	(0)	(0.16)	0.04*
Cco-27	AF453314	F: TGAATAATAACAAGCCTGAGATAAAGAT	55	125–149	8	0.31	0.49	0*
$(GAA)_7$		R: ACTACTGCCTAATGTAACCTCTAC		(119–134)	(3)	(0.40)	(0.48)	(0.33)
Cco-42	AF453315	F: AACAGATGCCTACTCTTATTCCA	60	91–97	3	0.03	0.06	0.02*
$(CTT)_6$		R: TAACACTACTGGCTGTATATACTCC		(94–100)	(3)	(0)	—	—
Cco-51	AF453316	F: CCACACAATCTACCAACGG	65	137–152	3	0.48	0.59	0.42
$(AGT)_{15}$		R: GCCTGATATAAACCGTCCATAT			NA	—	—	—
Cco-65A	AF453317	F: CAACTCGGACAACGCCTGCG	60	142–163	8	0.4	0.69	0*
$(CAA)_9(CTA)_{11}$		R: CCTCTCTGGCTAGTGGCATGGA		(166–181)	(5)	(0.5)	(0.66)	(0.24)
Cco-65B	AF453317	F: GGTTAGTATTATTTGATTTTTGGTCCTTAGA	55	151–190	6	0.26	0.34	0.01*
$(ACC)_2(AGC)_2(ACC)(AAC)_3$		R: TGTTACAGGCTCTTTCAGGG		(175–181)	(2)	(1.0)	(1.0)	—
$(AGC)_2(AAT)(ACT)(AGC)_{11}$								
Cco-68	AF453318	F: GAGGGCGAGAAGAGCAAC	60	153–198	15	0.74	0.9	0*
$(GAC)_9(GAT)_8$		R: AGCCATTTTGCCTCCCC		(162)	(1)	(0)	—	—

T_a, annealing temperature; SR, observed allele size range; A, observed numbers of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE, P-value for exact test of fit to Hardy–Weinberg equilibrium (significant departure is shown by an asterisk *); NA, no amplification product. — indicates data unavailable for calculation.

Table 2 Attributes of nine microsatellite loci examined in six species of *Cotesia* other than *C. congregata*. L, indicates method of fluorometric resolution (F: forward primer, R: reverse primer, C: dCTPs); T_a, annealing temperature; SR, observed allele size range; A, observed numbers of alleles. Species designations are: *C.a.*: *Cotesia acuminata*, *C.b.*: *C. bignellii*, *C.c.*: *C. cynthiae*, *C.e.*: *C. euphydryidis*, *C.k.*: *C. koebelei*, *C.m.*: *C. melitaearum*

			Species											
			C.a.		*C.b.*		*C.c.*		*C.e.*		*C.k.*		*C.m.*	
Locus	L	T_a (°C)	SR	A	SR	A	SR	A	SR	A	SR	A	SR	A
Cco-1A	F	55	127–154	4	127–148	3	133	1	136–142	2	136–139	2	106–145	9
Cco-5A	F	56	87–90	2	93–108	3	108	1	96	1	84–87	2	99–117	4
Cco-5B*	C	56	110–115	2	110–115	2	110–115	2	110–115	2	110–115	2	110–115	2
Cco-27	R	56	110	1	113–116	2	186	1	110	1	122–128	2	122–158	9
Cco-42	F	56	89–122	4	113–122	4	122	1	104–122	3	110–113	2	89–122	9
Cco-51	C	52	NA	0	NA	0	NA	0	NA	0	NA	0	NA	0
Cco-65A	F	57	140–170	8	152–164	6	131–134	2	110	1	146–158	3	128–173	12
Cco-65-B	F	55	125–137	3	125–134	2	169	1	0	0	137	1	116–149	10
Cco-68	F	55	154–160	3	154–163	4	157	1	163–166	2	145–151	3	154–172	5

*This locus was monomorphic for *C. congregata*.

Of the 14 repeat sequences from *C. congregata* examined, eight contained complex repeats [e.g. $(CTG)(TTG)_2$ $CTG(TTG)_2(CTG)_8(TTG)_{16}(CTG)$], and two were unusually long (> 40 tandem motif repeats). Of nine selected loci, all amplified products were in the size ranges expected from insert sequences. One locus yielded homologous products and eight were polymorphic (Table 1). A 52-bp segment of Cco-51 (≈ 300 bp upstream of the microsatellite) shared

homology with the polyDNA virus that is integrated into the *C. congregata* genome (GenBank accession number AF049877). All polymorphic loci exhibited Mendelian segregation. Allelic distributions differed significantly between the solanaceous and catalpa groups ($P = 0$, Fisher's exact tests for all loci) lending additional support to the hypothesis that host plant chemistry is a significant factor influencing the population structure of this species. Allele size ranges at one locus (Cco-65A) were different for the solanaceous and catalpa groups. Two pairs of loci demonstrated linkage disequilibrium in the solanaceous group (Cco-1A, Cco-27, $P = 0.01$; Cco-5A, Cco-65A, $P = 0.03$). Locus 51 conformed to Hardy–Weinberg Equilibrium (HWE) for the solanaceous group ($P = 0.42$), Cco-65A conformed to HWE for the catalpa group ($P = 0.24$), and Cco-27 conformed to HWE for the catalpa group ($P = 0.33$); the remainder did not conform to HWE ($P < 0.05$). Heterozygosity for polymorphic loci was lower than expected, suggesting the common occurrence of segregating null alleles. When tested for cross-reactivity to congeners, all but Cco-51 yielded various polymorphic products (Table 2). For the cross-species tests, loci Cco-42, Cco-65A, Cco-65B and Cco-68 yielded allele sizes that differed substantially from the sizes observed for *C. congregata*.

Acknowledgements

We thank A. Jensen, E. Freeman, A. Lentz, D. Franklin, K. Bleecher, G. Lathrop and K. Livingstone for their assistance and comments. This research was facilitated by a grant from the Jeffress Memorial Trust (J-513) and by Ecological Genetics, Inc.

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