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Isolation and characterization of polymorphic microsatellite loci in two primary parasitoids of the noctuid *Spodoptera frugiperda*: *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera)

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Abstract

Fifteen and 13 microsatellite loci were isolated, respectively, from *Campoletis sonorensis* Cameron and from *Chelonus insularis* Cresson. These two parasitic Hymenoptera are primary parasitoids of Lepidoptera in North, Central and South America, including the important agricultural pest *Spodoptera frugiperda*. Allelic diversity and heterozygosity were quantified in samples from Mexico. Each locus was polymorphic, with the number of alleles ranging from two to 16 in *C. sonorensis* and from four to 18 in *C. insularis*. Heterozygosity ranged from 0.088 to 0.403 in *C. sonorensis* and from 0.106 to 0.458 in *C. insularis*.

Keywords

Campoletis, *Chelonus*, microsatellites, parasitoids, population structure

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is one of the most devastating pests in the Americas (Kranz *et al*. 1977), attacking numerous crops of great economic importance (Sparks 1979; Knipling 1980; Pashley 1986). This Lepidoptera is in turn attacked by a number of parasitoid species (Molina-Ochoa *et al*. 2000, 2003, 2004). A good understanding of the patterns of genetic variability within and among parasitoid populations may help us to determine the selection forces that drive their population dynamics and evolution. *Chelonus insularis* Cresson (Hymenoptera: Braconidae: Cheloninae) is an egg-larval parasitoid of *Spodoptera* sp. while *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae: Campoleginae) is a larval parasitoid. Both species are distributed over South, Central and North America and they were the two predominant parasitoid species collected in Mexico during the summers of 2005 and 2006. Microsatellite markers were developed to investigate a potential effect of the host plant on the population genetic structure of parasitoids.

Total genomic DNA was isolated from four individuals of *C. insularis* and from three individuals of *C. sonorensis*, using the DNeasy Tissue Kit (QIAGEN) following the manufacturer's instructions. Enriched DNA library with microsatellite sequences and sequencing of 192 clones per species were performed by the SREL DNA Laboratory, University of Georgia, USA following the protocol of Glenn & Schable (2005). Cloned microsatellite sequences were edited using the ChromasPro 1.41 software (Technelysium Pty Ltd). Microsatellite loci which offered a flanking region long enough to design primers were selected. Seventy-six and 98 primer pairs were then designed, respectively, for *C. insularis* and *C. sonorensis* with Primer 3 (version 0.4.0; Rozen & Skaletsky 2000) using stringent conditions [i.e. primer melting temperature (T_m): 50–60 °C (optimal: 55 °C); maximum T_m difference: 1 °C; GC content: 40–60%; maximum self-complementarity: 6; maximum 3′ self-complementarity: 2; maximum poly-X: 4; GC clamps: 2].

Polymerase chain reaction (PCR) amplifications were carried out in 10-μL volumes containing 1 μL of extracted DNA, 1.5 mM $MgCl_2$ depending on the locus (Table 1), 1 μL of 1.5 mM dNTPs, 1 μL of PCR buffer (Promega), 1 U of *Taq* DNA polymerase (Promega), 0.5 μL of 10 μM forward primer, and 0.5 μL of 10 μM reverse primer. Microsatellites were amplified in Biometra *Uno*II thermocyclers (Biometra) using the following touchdown programme: initial denaturation at 94 °C for 1 min 30 s; five cycles of 94 °C for 45 s, 60 °C for 45 s, 70 °C for 45 s; five cycles of 94 °C for 45 s,

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Table 1 Attributes of the *Campoletis sonorensis* and *Chelonus insularis* microsatellite loci: *N* indicates the number of females scored for each species, N_a the number of alleles, and T_m the melting temperature of the primer pair. Results of Hardy-Weinberg exact test (HW) comparing observed (H_O) and expected (H_E) heterozygosities are reported

Locus	Repeat	Size range (bp)	N_a	Primer sequence (5′–3′) (F, forward; R, reverse)	Fluorescent dye	[$MgCl_2$] (mM)	T_m (°C)	H_E	H_O	HW†	GenBank Accession no.
Campoletis sonorensis $N = 68$											
Cs6	$(TAGA)_6$	230–258	8	F: CGAGAATCGAGAGACACG R: GAACATTCGCGTGAGC	6-FAM	1.5	55	0.348	0.279	**	EU678871
Cs9	$(TCTA)_{10}$	98–122	6	F: GCATTCTCACGCATCG R: AGCGAAACACCTTACCG	6-FAM	1.5	55	0.284	0.228	NS	EU678872
Cs14	$(CTT)_5$	150–162	5	F: AGCCACGTAATACAAAGTCG R: TGTGTGTGTCAGAGGATCG	6-FAM	1.5	55	0.289	0.321	NS	EU678873
Cs15	$(TG)_{11}$	149–165	5	F: AGAGGAAGCTCTGTGACG R: AGCTCAACCCACTCTCG	6-FAM	1.5	54	0.136	0.156	NS	EU697897
Cs19	$(TGA)_{21}$	235–279	16	F: CTGGCATTGTGTGTCG R: CTTCTAACTTTGCGTCTCG	6-FAM	1.5	53	0.459	0.403	*	EU697898
Cs20	$(TGA)_8$	209–227	7	F: GAGTGTCTGCGTGAAAGG R: GATCCGTTTATCGTGTATCG	NED	1.5	55	0.355	0.324	NS	EU678874
Cs21	$(GA)_{10}$	214–254	16	F: ATGGAAGCTGGTTGAAGG R: CCTCATCATCGTCTGTGG	NED	1	55	0.400	0.338	***	EU678875
Cs22	$(GA)_8$	232–264	11	F: ACGATCACGAGACAGTGAG R: TATTTGTGTGCCATGGTG	NED	1.5	54	0.334	0.375	NS	EU678876
Cs23	$(CAAA)_6$	178–198	6	F: GATAGCTGCACGAAAACG R: GATACCTGTCTCGCTATTGG	HEX	1.5	55	0.173	0.144	*	EU697899
Cs37	$(TTGA)_8$	92–104	4	F: CATAACCGAGAGGAATCG R: GCCCTAAGGACTTATCCAG	6-FAM	1.5	54	0.276	0.161	***	EU697900
Cs42	$(CAAA)_6$	153–165	4	F: GAGTGTCACCCTGTCTCG R: GATGTTGATGATTAATTGTTGC	NED	1	55	0.281	0.294	NS	EU678877
Cs44	$(TGA)_5$	209–212	2	F: TGGGGTTGGTATATTTGG R: TGTTGGGTGATAGTTCAGG	HEX	1.5	54	0.206	0.213	NS	EU678878
Cs47	$(GTCT)_4$	204–216	3	F: CAACACGTGCCAATCG R: AATGGCTTTTCTCCAAGC	HEX	1.5	56	0.083	0.088	NS	EU678879
Cs48	$(GTT)_7$	150–174	8	F: CCAATGAGGTCAGTTTCG R: ACGAATGAACCACAGAGC	6-FAM	1.5	55	0.373	0.368	NS	EU678880
Cs49	$(TGAG)_4$	221–237	5	F: AAAACCACCAACTACTACGG R: TGCTAAAGCGAGAAAAGC	HEX	1	55	0.175	0.140	NS	EU678881
Chelonus insularis $N = 59$											
Ci1	$(CAA)_{26}$	304–373	18	F: GGCTTCCTGAATCAAATG R: GACAATGGTGAATTTGGAC	6-FAM	1	54	0.451	0.415	NS	EU678882
Ci9	$(TGTA)_{16}$	298–350	12	F: CCAGTGGAAAAGTATCG R: TGAGAACAGAATAGTTTAAGG	6-FAM	1	49	0.435	0.226	***	EU697893
Ci10	$(TGA)_7$	165–174	4	F: CAAAAGACGAGTATTGATGG R: CCAGCATAAGTTGGAGAAG	6-FAM	1.5	54	0.245	0.237	NS	EU678883
Ci11	$(CAA)_{11}$	182–212	9	F: ATCGACGTCAACATACTGG R: TGAAGGTGGTGGTAGTGG	NED	1	55	0.394	0.415	NS	EU678884
Ci12	$(TGA)_9$	247–265	7	F: TGCCTAGGTCATTTGC R: TGAGTTAATACTCTCGCTTG	6-FAM	1	51	0.331	0.345	NS	EU678885
Ci13	$(GT)_{14}$	261–279	6	F: CCTTCGATTATTCCTTTCG R: CCTTTGAAACCTGTAGATAACC	6-FAM	1	55	0.294	0.106	***	EU697894
Ci15	$(GTT)_7$	164–173	4	F: TGATTTGGTTGCTCTTGC R: TCCTCGAACTCTTCACACC	6-FAM	1.5	56	0.257	0.167	*	EU697895
Ci16	$(GA)_9$	214–228	5	F: TAGCTACATTGGGGATCG R: GGGTGAAATTCCCTTCC	NED	1.5	55	0.183	0.195	NS	EU678886
Ci17	$(TCA)_7$	187–208	7	F: GAAGCAAAGGGCAACG R: GCGAAGGACATACTCATGG	HEX	1	57	0.375	0.356	*	EU678887
Ci28	$(TGA)_{21}$	251–314	18	F: CAGACTGGTTGGTTACAGG R: AGCAGAATCACCAACAGC	6-FAM	1	54	0.459	0.420	*	EU697896
Ci30	$(GA)_{11}$	225–233	5	F: AGCCCAAGCAATATTATCC R: CTTTCTTCGTCATTCAGTCC	NED	1.5	55	0.309	0.328	NS	EU678888
Ci31	$(CT)_{15}$	256–284	12	F: CCTTAATTGTATTAGGCTCTGG R: GCCAATTGTCTGAAAAGC	6-FAM	1.5	55	0.436	0.458	NS	EU678889
Ci33	$(GA)_9$	167–173	4	F: TGGTGAGTGTGTGTGAGC R: GTATGAGCGGACAAAAGG	6-FAM	1.5	54	0.173	0.203	NS	EU678890

†NS, nonsignificant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

57 °C for 45 s, 70 °C for 45 s; 10 cycles of 94 °C for 45 s, 55 °C for 45 s, 70 °C for 45 s; 20 cycles of 94 °C for 45 s, 52 °C for 45 s, 70 °C for 45 s; final elongation at 70 °C for 5 min. PCR products were run on 0.6% agarose gels to check for amplification. These first tests were carried out on four females coming from the same population and on three females coming from three different locations. Among the 76 and 98 loci tested for the two species, respectively, only 13 and 15 provided satisfactory amplification (amplified bands of expected size with few supernumery bands), respectively, for *C. insularis* and *C. sonorensis.* Amplifying loci were then genotyped to check for polymorphism, eventually adjusting PCR conditions by reducing $MgCl_2$ concentration to 1 mM to eliminate supernumery bands preliminarily observed at some loci. For all loci, the forward primer was labelled with either 6-FAM, HEX or NED. Loci were multiplexed by size and by colour when possible to decrease the number of genotyping reactions. Genotyping of PCR products was performed on an ABI PRISM 3100 sequencer (Applied Biosystems) by Macrogen Inc. on 59 and 68 females (males were not genotyped because of the haplodiploid nature of inheritance in Hymenoptera), respectively, for *C. insularis* and *C. sonorensis*, from one population collected in the state of Guanajuato, Mexico (Table 1). Genotypes were scored using Peak Scanner Software version 1.0 (Applied Biosystems). All loci were then checked for the presence of null alleles using Micro-Checker (Van Oosterhout *et al*. 2004). Eight loci showed evidence for the presence of null alleles (*Ci9*, *Ci13*, *Ci15*, and *Ci28* in *C. insularis*, and *Cs19*, *Cs21*, *Cs23* and *Cs37* in *C. sonorensis*).The expected heterozygosity and the observed heterozygosity were calculated using GenAlEx (available at http://www.anu.edu.au/BoZo/GenAlEx/; Peakall & Smouse 2006). The number of alleles, genotypic disequilibrium and deviation from Hardy-Weinberg equilibrium (HWE) were estimated using GenePop 4.0 (Raymond & Rousset 1995).

Allelic diversity ranged from four to 18 alleles in *C. insularis* and from two to 16 alleles in *C. sonorensis*. Heterozygosities were between 0.106 and 0.458 in *C. insularis* and between 0.088 and 0.403 in *C. sonorensis*. Five loci showed significant deviation from HWE in both *C. insularis* and *C. sonorensis* when exact tests (GenePop 4.0; Raymond *et al.* 1995) were performed. No linkage disequilibrium was observed for any loci pair in *C. insularis*. In *C.* sonorensis, the exact test for genotypic disequilibrium showed four loci pairs with significant *P* values after Bonferroni corrections: *Cs15-Cs19* ($P < 0.01$), *Cs19-Cs21* ($P < 0.01$), *Cs-19-Cs48* ($P < 0.01$) and *Cs20-Cs21* ($P < 0.01$).

These results showed that the microsatellite DNA loci described here are highly polymorphic, and that these markers will be useful in the investigation of population genetic structure, parentage and mating system of *C. insularis* and *C. sonorensis*. Having reliable molecular tools at hand is one of the keys to a good understanding of the interactions between parasitoids and their hosts, and can help to develop novel strategies in managing populations of major pests, such as *S. frugiperda*.

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