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Microsatellite markers in a complex of *Horismenus sp.* (Hymenoptera: Eulophidae), parasitoids of bruchid beetles

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Abstract

Parasitoids of the genus *Horismenus* (Hymenoptera: Eulophidae) are the main natural enemies of bruchid beetles that feed on several species of *Phaseolus* beans. Samples of *Horismenus depressus*, *H. missouriensis* and *H. butcheri* were collected from seeds of three *Phaseolus* species throughout Mexico to examine the impact of plant variability on the genetic structure of parasitoid populations. For this purpose, six microsatellite loci were isolated and characterized. These loci are of great interest in understanding the taxonomy of the genus *Horismenus*, the most important Eulophid genus in the Neotropics.

Keywords: beans, Eulophidae, *Horismenus*, microsatellite, parasitoid, population structure

Host location by parasitoids and their reproductive success can be greatly influenced by the plant species on which the host feeds. Various studies show that features of plants, such as allelochemistry, nutritional quality and morphology, can affect natural enemies directly or indirectly (Barbosa & Benrey 1998; Benrey *et al*. 1998; Turlings & Benrey 1998). As yet, little is known about how these plant effects influence the genetic population structure of the parasitoids.

Bruchid beetles that feed on seeds of the genus *Phaseolus* are attacked by several species of hymenopteran parasitoids. We use this system to investigate the role of plants on the genetic population structure of a complex of parasitoids. For this purpose, we collected individuals of the genus *Horismenus*, the most important parasitoid in this system, from bruchid-infested beans (*P. vulgaris*, *P. coccineus* and *P. lunatus*) throughout Mexico. Preliminary genetic data suggested the presence of various species in the original sample. Based on morphological traits, three species were identified: *Horismenus depressus* Gahan, *H. missouriensis* Ashmead and *H. butcheri* Hansson and Aebi (Hansson *et al*. 2004).

Total genomic DNA was extracted using a Puregen™ DNA isolation kit (Gentra Systems) from a pool of 136 individuals (0.135 g of fresh material) of the three species, collected in Tepoztlan, Tejupilco and Temascaltepec (Mexico State). Microsatellite-enriched libraries were built following Billotte *et al*. (1999): DNA was digested to completion with *Rsa*I (Eurogentec) and 500–1000 bp DNA fragments were selected after resolving on TAE-0.8% (w/v) agarose gel and isolated using an extraction kit (Promega). The enrichment step was pursued as described in Kijas *et al*. (1994) and Edwards *et al*. (1996). The enriched microsatellite partial library was then constructed by ligating the polymerase chain reaction (PCR) products into pGEM-T (Promega), following the manufacturer's recommendations. Epicurian-coli XL1-Blue MRF' supercompetent cells (Stratagene) were used for the transformation of the cloned DNA fragments. Following standard blue–white selection on Xgal/IPTG/ampicilin plates, 384 white transformant clones were transferred on Hybond-N+ nylon membranes (Amersham) and hybridized using ^{32}P-labelled oligoprobes $(CT)_{15}$ and $(GT)_{15}$. After hybridization, the filters were washed twice with 4× SSC (10 min, 57 °C) and then with 0.1× SSC/10% (w/v) SDS (10 min, 40 °C). Of these clones, 223 gave a strong positive signal, of which 48 inserted DNA fragments were sequenced. Fourteen primer pairs were designed of which six gave satisfactory amplification patterns (i.e. PCR products of the predicted size, and supernumerary bands of low intensity).

PCR amplifications were performed in a final volume of 5 μL containing 1 μL of extracted DNA (2 ng/μL), 2.5 μL of HotStarTaq Master Mix (Qiagen), 0.25 μL of 10 μM reverse

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Table 1 Polymorphic microsatellite loci for *Horismenus depressus*

Locus	Motif	Genbank Accession no.	Primer sequences (5′–3′)	Size range (bp)	T_a (°C)	Atila (n = 18)			Temascaltepec (n = 9)		
						Na	H_E	H_O	*Na*	H_E	H_O
Ho4b	$(GT)_9$	AY166613	F: CATCGAAAGGGATATGCGCACG R: CTATACAAAGCTCCATTCACTCG	126–140	61	6	0.60	0.72	6	0.64	0.33*
Ho8b	$(CA)_6TA(CA)_{16}$	AY655757	F: CTTAAAACTCTACAATGGCGTCTTT R: GATAAAGTACAGATTTCGCGC	164–298	58	9	0.71	0.44*	10	0.91	0.56*
Ho10b	$(CT)_{11/5}$	AY166616	F: GCATAGAGTCGCGGAATCG R: CCACTCGAAATACTTGTAAC	154–174	63	8	0.62	0.61	5	0.78	0.78
Ho16	$(GA)_{49}$	AY166618	F: TCTGAACTTGCATTGTCATG R: GCAAAATTGCGTTTCGTCTG	170–238	57	15	0.85	0.78	7	0.43	0.44
Ho6b	$(GA)_{22}$	AY166614	F: CGTTATGCGCATACGCTGGGT R: CAACACAAGACAACGCAGCTCCG	163–181	65	6	0.62	0.50	7	0.86	0.67
Ho9b	$(CT)_9$	AY655758	F: TGTGCGTGGTATATGGCTCAC R: AGGACGATCGATTCCGCGAC	95–113	60	5	0.68	0.50*	4	0.73	0.11*

T_a: annealing temperature, *Na*: number of alleles detected, H_E: expected heterozygosity under Hardy–Weinberg equilibrium, H_O: observed heterozygosity, n: number of individuals tested. *Significant deviation from H_E ($P < 0.05$).

Table 2 Polymorphic microsatellite loci for *Horismenus missouriensis* and *H. butcheri*

	Horismenus missouriensis							*Horismenus butcheri*						
		Malinalco (n = 16)			Temascaltepec (n = 11)				Malinalco (n = 23)			Temascaltepec (n = 18)		
Locus	T_a (°C)	*Na*	H_E	H_O	*Na*	H_E	H_O	T_a (°C)	*Na*	H_E	H_O	*Na*	H_E	H_O
Ho4b	61	9	0.71	0.44*	4	0.68	0.55*	61	—	—	—	—	—	—
Ho8b	58	19	0.85	0.75	8	0.73	0.73	58	17	0.58	0.48	5	0.38	0.22*
Ho10b	60	6	0.72	0.38*	3	0.41	0.09*	62	8	0.59	0.52	7	0.51	0.44
Ho16	57	15	0.83	0.75	12	0.78	0.73	57	19	0.79	0.83	19	0.75	0.77
Ho6b	65	7	0.71	0.50*	5	0.46	0.45	65	11	0.71	0.39*	2	0.06	0.06
Ho9b	60	8	0.78	0.31	5	0.67	0.64	60	10	0.83	0.74	6	0.45	0.39

Abbreviations as in Table 1.

and forward primer (5′ IRD-700/800 modified) and 1 μL of double-distilled H_2O. PCRs were performed on a Biometra® T gradient thermocycler using the following cycling conditions: initial denaturing step of 95 °C for 15 min followed by 40 cycles of: 95 °C (30 s), T_a (30 s), 72 °C (40 s); final elongation at 72 °C (10 min). PCR products were mixed with 2.5 μL of stop solution [95% (v/v) desionized formamide, 50 mM EDTA/10 mM NaOH/0.1% (w/v) bromophenol blue/0.1% (w/v) xylene cyanol green] and denatured at 94 °C for 2 min, prior to electrophoresis on a denaturing 7.4 M urea –6.5% (w/v) polyacrylamide gel (Sequagel *XR*, National Diagnostics) on a Li-Cor DNA Analyser. Isolated bands were visualized and analysed using SAGA IR² software, version 2.2.2.

The degree of polymorphism at the six loci was tested for each *Horismenus* species using females from two different populations. We calculated expected and observed heterozygosities, estimated heterozygote deficit using Hardy–Weinberg exact tests and checked for linkage desequilibrium (GENEPOP 3.3; Raymond & Rousset 1995).

For *H. depressus*, all loci were polymorphic, with 4–15 alleles per population (Table 1). A significant deficit of heterozygotes was observed for three loci (*Ho8b* and *Ho9b* in both populations and *Ho4b* in Temascaltepec), suggesting the presence of null alleles. Eight of the 15 possible pairs of loci were out of linkage disequilibrium in Atila, whereas none was out of linkage disequilibrium in Temascaltepec. The inconsistency between sites suggests a Wahlund effect in Atila, a large population growing on *P. vulgaris* with the peculiarity of being close to populations of parasitoids growing on *P. lunatus*, thereby potentially creating substructure in the population.

We found 3–19 alleles per locus in *H. missouriensis* (Table 2). A significant deficit of heterozygotes was observed for five

loci (*Ho4b*, *Ho10b* in both populations and *Ho8b*, *Ho6b*, *Ho9b* in Malinalco), suggesting the presence of null alleles. No linkage-disequilibrium was detected.

We found 2–19 alleles in *H. butcheri* (Table 2). A significant deficit of heterozygotes was seen for two loci (*Ho6b* in Malinalco and *Ho8b* in Temascaltepec) suggesting the presence of null alleles. No linkage disequilibrium was detected.

PCR conditions were optimized for each species separately (Tables 1 and 2). All loci except for *Ho4b* in *H. butcheri* showed a high degree of cross-amplification, providing useful tools for population genetics studies in this *Horismenus* complex. The genus *Horismenus* is one of the largest in the Eulophidae in the Neotropics but the majority of species remain undescribed (Hansson *et al*. 2004). These microsatellites provide valuable tools for a better knowledge of the Neotropical *Horismenus*.

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