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Isolation and characterization of microsatellites in the endoparasitic ichneumonid wasp carrying a polydnavirus *Hyposoter didymator*

Philippe Audiot · Véronique Jouan ·
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Abstract The wasp *Hyposoter didymator* (Hymenoptera, Ichneumonidae) parasitizes several agricultural pest moths and could therefore be used in biological control. The 454 FLX Titanium pyrosequencing technology was used to define two distinct sets of multiplex combining 14 polymorphic microsatellite loci: 10 (referred to as HD) located within the genome of *H. didymator* and 4 (referred to as HdIV) located within the Ichnovirus genome which is integrated into the wasp genome. Genotyping of two populations collected in France on *Helicoverpa armigera* revealed that most of the loci are independent and at Hardy–Weinberg equilibrium.

Keywords *Hyposoter didymator* · *Helicoverpa armigera* · Pyrosequencing · Parasitoid

Hyposoter didymator is a larval endoparasitoid wasp of many Lepidoptera larvae that carries a symbiotic polydnavirus (named *H. didymator* Ichnovirus, HdIV) integrated into its genome, insuring, like many other wasps, its vertical transmission. The most common host of *H. didymator* is the moth *Helicoverpa armigera* (Lepidoptera: Noctuidae), but this wasp can also parasitize other noctuid pest species. *H. didymator* may partly regulate their

populations and therefore can be used in biological control of those pests.

Microsatellite markers of this parasitoid were isolated using the new generation 454 FLX titanium pyrosequencing technology (Malausa et al. 2011). Enrichment of microsatellite loci was carried out at Genoscreen (Lille, France) using the procedure described by Clamens et al. and Dumas et al. (in Arias et al. 2012). The selection of 454 FLX Titanium sequences for primer design was done using QDD software (Meglécz et al. 2010).

A total of 1,290 microsatellites loci were identified, amongst which 298 allowed designs of PCR amplification primers. Based on the expected sizes of amplification products, first attempts for polymorphism, multiplexing and quality of the chromatograms (details not shown), we chose 14 polymorphic microsatellite loci: 10 HD (referred to as HD loci) were located within the genome of *H. didymator* and 4 (referred to as HdIV) were located within the HdIV genome (Table 1). Those 14 loci were then amplified in two multiplex (M1 and M2) PCRs. PCRs were conducted in 10 µL reaction volume containing the Qiagen Multiplex PCR Master Mix (1×)—with a final concentration of 3 mM of MgCl₂ and an annealing at 58 °C for 1.5 min.

To assess the polymorphism at these 14 loci, we genotyped 10 males and 19 females from Notre-Dame-de-Londres (NDL, 43°49'N, 3°46'E, France) and 28 males and 42 females from Mauguio (43°37'N, 4°00'E, France). Each individual originated from an *H. armigera* larva collected on alfalfa (*Medicago sativa* L.). DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). All microsatellite loci displayed at least 4 alleles per locus (Table 1).

As expected, at all loci, all males (n = 38) displayed only one allele, whereas all females (n = 61) displayed two different alleles—i.e., were heterozygotes—at least at

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Table 1 Characteristics of loci isolated in *Hyposoter didymator*, with primer sequences, size of cloned allele, number of alleles (No), size range of PCR products, *Fis*

Locus	Primer sequence (5'–3')	Repeat motif	SetNo— PCR	Size (bp)	Total No	NDL (France)		Mauguio (France)		GenBank accession no.
						No	Size range	No	Size range	
HD42	F: FAM-CAGCCTTTCTCTATTTTATCTCCA	(CT) ₉	M2	092	7	5	086–094	7	086–100	0.147 0.000 KF226121
	R: CTTGGCGAAATGCAGGAG									
HD43	F: VIC-CGAGAGTCAACATTCGCCTT	(ATC) ₉	M2	110	4	3	110–116	4	105–116	–0.100 0.000 KF226122
	R: GATGATCGTTGTGATCCTCG									
HD47	F: PET-AAATGCAACTAACTGGGTGT	(CGT) ₉	M2	099	5	4	093–105	5	093–105	0.222 0.014 KF226123
	R: ATGACGTTAACAACTACGAA									
HD59	F: NED-AGAAACAACCCCTGAAATGG	(ACA) ₅	M1	170	4	3	164–170	4	164–173	–0.138 0.000 KF226124
	R: TCCTATCTTGACGTGGAGGG									
HD65	F: FAM-GATCGTCGTCCTTGTGCTTT	(TCG) ₆	M1	282	4	4	276–285	4	276–285	0.072 0.000 KF226125
	R: CAGACCGAGACTGGAAGGAG									
HD70	F: NED-GCAACAGCAACGGCAATA	(AGC) ₆	M2	159	4	3	159–165	4	156–165	–0.010 0.000 KF226126
	R: GTGTACGAATCGCTGGGACT									
HD77	F: FAM-CTCTTGTCAAACGCCACAAA	(CAA) ₈	M1	230	5	4	227–236	5	277–239	–0.049 0.000 KF226127
	R: GGTAAATGGTCTCGTTGCGAT									
HD80	F: NED-TACTCCACATTACCACCGC	(CA) ₈	M1	188	10	7	186–202	9	184–200	–0.020 0.000 KF226128
	R: CATCAACTCGCTGTGGGATA									
HD81	F: PET-GTGGTCAGCTACGGAAAGAGC	(AG) ₉	M2	192	11	5	163–195	11	163–198	0.061 0.028 KF226129
	R: CAGCGTGCATCTTCGTGTTA									
HD90	F: VIC-CAITGTGTTTGTCTGGGG	(GTT) ₉	M1	154	7	4	148–157	7	145–163	0.004 0.000 KF22630
	R: GGCTTTGTCTGGTTATGGGA									
HdIV6	F: FAM-GATTGGGGTCATTGTATGGT	(TA) ₈	M2	386	4	3	382–386	4	382–388	–0.051 0.000 KF22631
	R: ATGAGTTGATAGCTGCCACA									
HdIV7	F: VIC-CTGACGCCACTGTACTTTG	(GT) ₉ TT(GT) ₃	M2	484	5	3	482–486	5	476–490	–0.052 0.000 KF22632
	R: AAAACCAACTGATTGTTCTGT									
HdIV8	F: FAM-ACGTGATGGATGTCAGTACG	(GT) ₅ AT(GT) ₈	M1	333	11	8	329–347	10	329–353	0.037 0.001 KF22633
	R: AGTTACCCCTATGACTCTGGCA									
HdIV9	F: PET-GGGACCCAAATGATCAACAG	(GT) ₉	M1	126	5	3	122–126	5	122–130	–0.068 0.000 KF22634
	R: GCCTCAACTGCTGCCAATTA									

Freq NA frequency of null alleles

six loci, which is consistent with *H. didymator* displaying the dominant mode of sex determination (females being diploid, males being haploid) in Hymenoptera (Heimpel and de Boer 2008).

Tests performed with GENEPOP 4.0 (Rousset 2008) revealed that the genotypic distribution did not significantly differ from those expected at Hardy–Weinberg equilibrium (HWE) except at NDL for one locus (Table 1). Accordingly, null allele frequencies were <3 % at all loci but one and in each population (Table 1). Across the two populations, three pairs of loci showed significant linkage disequilibrium ($P < 0.001$ after multiple testing correction): HD43 and HD77, HD77 and HD90 and HD47 and HdIV8.

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