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

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

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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 $MgCl_2$ 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′) Include fluro-label dye	Repeat motif	SetNo—PCR	Size (bp)	Total No	NDL (France)				Mauguio (France)				GenBank accession no.
						No	Size range	*Fis*	Freq NA	No	Size range	*Fis*	Freq NA	
HD42	F: FAM-CAGCCTTTCTCTATTTTTATCTCCA R: CTTGCGCAAATGCAGGAG	$(CT)_9$	M2	092	7	5	086–094	0.066	0.000	7	086–100	0.147	0.000	KF226121
HD43	F: VIC-CGAGAGTCAACATTCGCCTT R: GATGATCGTTGTGATCCTCG	$(ATC)_9$	M2	110	4	3	110–116	0.055	0.016	4	105–116	−0.100	0.000	KF226122
HD47	F: PET-AAATGCAACTAACTGGGTGT R: ATGACGTTAACAACTACGAA	$(CGT)_9$	M2	099	5	4	093–105	0.128	0.021	5	093–105	0.222	0.014	KF226123
HD59	F: NED-AGAAACAACCCCTGAAATGG R: TCCTATCTTGACGTGGAGGG	$(ACA)_5$	M1	170	4	3	164–170	0.190	0.000	4	164–173	−0.138	0.000	KF226124
HD65	F: FAM-GATCGTCGTCCTTGTCGTTT R: CAGACCGAGACTGGAAGGAG	$(TCG)_6$	M1	282	4	4	276–285	−0.323	0.000	4	276–285	0.072	0.000	KF226125
HD70	F: NED-GCAACAGCAACGGCAATA R: GTGTACGAATCGCTGGGACT	$(AGC)_6$	M2	159	4	3	159–165	−0.362	0.000	4	156–165	−0.010	0.000	KF226126
HD77	F: FAM-CTCTTGTCAAACGCCACAAA R: GGTAATGGTCTCGTTGCGAT	$(CAA)_8$	M1	230	5	4	227–236	−0.161	0.000	5	277–239	−0.049	0.000	KF226127
HD80	F: NED-TACTCCCACTTTACCACCGC R: CATCAACTCGCTGTGCGATA	$(CA)_8$	M1	188	10	7	186–202	0.470	0.127	9	184-200	−0.020	0.000	KF226128
HD81	F: PET-GTGGTCAGCTACGGAAGAGC R: CAGCGTGCATCTTCGTGTTA	$(AG)_9$	M2	192	11	5	163–195	0.127	0.026	11	163–198	0.061	0.028	KF226129
HD90	F: VIC-CATTGTTGTTTTGCTGGGG R: GGCTTTGTCTGGTTATGGGA	$(GTT)_9$	M1	154	7	4	148–157	0.023	0.000	7	145–163	0.004	0.000	KF22630
HdIV6	F: FAM-GATTGGGGTCATTGTATGGT R: ATGAGTTGATAGCTGCCACA	$(TA)_8$	M2	386	4	3	382–386	−0.113	0.000	4	382–388	−0.051	0.000	KF22631
HdIV7	F: VIC-CTGACGCCACTGTTACTTTG R: AAACCCAACTGATTGTTCGT	$(GT)_9TT(GT)_3$	M2	484	5	3	482–486	0.161	0.000	5	476–490	−0.052	0.000	KF22632
HdIV8	F: FAM-ACGTGATGGATGTCAGTACG R: AGTTACCCTATGACTCTGGCA	$(GT)_5AT(GT)_8$	M1	333	11	8	329–347	0.080	0.000	10	329–353	0.037	0.001	KF22633
HdIV9	F: PET-GGGACCCAAATGATCAACAG R: GCCTCAACTGCTGCCAATTA	$(GT)_9$	M1	126	5	3	122–126	−0.045	0.000	5	122–130	−0.068	0.000	KF22634

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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References

Arias MS et al (2012) Permanent genetic resources added to molecular ecology resources database 1 December 2011-31 January 2012. Mol Ecol Resour 12:570–572

Heimpel GE, de Boer JG (2008) Sex determination in the Hymenoptera. Annu Rev Entomol 53:209–230

Malausa T, Gilles A, Meglécz E et al (2011) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. Mol Ecol Resour 11:638–644

Meglécz E, Costedoat C, Dubut V, Gilles A, Malausa T, Pech N, Martin JF (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403–404

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103–106