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

Philippe Audiot - Véronique Jouan -<br>Marie Frayssinet • Anne-Nathalie Volkoff •<br>Denis Bourguet


#### 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 $\cdot$ 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

[^0]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 $10 \mu \mathrm{~L}$ reaction volume containing the Qiagen Multiplex PCR Master Mix ( $1 \times$ )-with a final concentration of 3 mM of $\mathrm{MgCl}_{2}$ and an annealing at $58{ }^{\circ} \mathrm{C}$ for 1.5 min .

To assess the polymorphism at these 14 loci, we genotyped 10 males and 19 females from Notre-Dame-deLondres (NDL, $43^{\circ} 49^{\prime} \mathrm{N}, 3^{\circ} 46^{\prime} \mathrm{E}$, France) and 28 males and 42 females from Mauguio ( $43^{\circ} 37^{\prime} \mathrm{N}, 4^{\circ} 00^{\prime} \mathrm{E}$, 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 $(\mathrm{n}=38)$ displayed only one allele, whereas all females ( $n=61$ ) displayed two different alleles-i.e., were heterozygotes-at least at
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 $\left(5^{\prime}-3^{\prime}\right)$ Include fluro-label dye | Repeat motif | $\begin{aligned} & \text { SetNo- } \\ & \text { PCR } \end{aligned}$ | $\begin{aligned} & \text { Size } \\ & \text { (bp) } \end{aligned}$ | 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 <br> R: CTTGCGCAAATGCAGGAG | $(\mathrm{CT})_{9}$ | M2 | 092 | 7 | 5 | 086-094 | 0.066 | 0.000 | 7 | 086-100 | 0.147 | 0.000 | KF226121 |
| HD43 | F: VIC-CGAGAGTCAACATTCGCCTT <br> R: GATGATCGTTGTGATCCTCG | $(\mathrm{ATC})_{9}$ | M2 | 110 | 4 | 3 | 110-116 | 0.055 | 0.016 | 4 | 105-116 | -0.100 | 0.000 | KF226122 |
| HD47 | F: PET-AAATGCAACTAACTGGGTGT <br> R: ATGACGTTAACAACTACGAA | $(\mathrm{CGT})_{9}$ | M2 | 099 | 5 | 4 | 093-105 | 0.128 | 0.021 | 5 | 093-105 | 0.222 | 0.014 | KF226123 |
| HD59 | F: NED-AGAAACAACCCCTGAAATGG <br> R: TCCTATCTTGACGTGGAGGG | $(\mathrm{ACA})_{5}$ | M1 | 170 | 4 | 3 | 164-170 | 0.190 | 0.000 | 4 | 164-173 | -0.138 | 0.000 | KF226124 |
| HD65 | F: FAM-GATCGTCGTCCTTGTCGTTT <br> R: CAGACCGAGACTGGAAGGAG | $(\mathrm{TCG})_{6}$ | M1 | 282 | 4 | 4 | 276-285 | -0.323 | 0.000 | 4 | 276-285 | 0.072 | 0.000 | KF226125 |
| HD70 | F: NED-GCAACAGCAACGGCAATA <br> R: GTGTACGAATCGCTGGGACT | $(\mathrm{AGC}){ }_{6}$ | M2 | 159 | 4 | 3 | 159-165 | -0.362 | 0.000 | 4 | 156-165 | -0.010 | 0.000 | KF226126 |
| HD77 | F: FAM-CTCTTGTCAAACGCCACAAA <br> R: GGTAATGGTCTCGTTGCGAT | $(\mathrm{CAA})_{8}$ | M1 | 230 | 5 | 4 | 227-236 | -0.161 | 0.000 | 5 | 277-239 | -0.049 | 0.000 | KF226127 |
| HD80 | F: NED-TACTCCCACTTTACCACCGC <br> 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 <br> 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 <br> R: GGCTTTGTCTGGTTATGGGA | $(\mathrm{GTT})_{9}$ | M1 | 154 | 7 | 4 | 148-157 | 0.023 | 0.000 | 7 | 145-163 | 0.004 | 0.000 | KF22630 |
| HdIV6 | F: FAM-GATTGGGGTCATTGTATGGT <br> 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 <br> R: AAACCCAACTGATTGTTCGT | $(\mathrm{GT})_{9} \mathrm{TT}(\mathrm{GT})_{3}$ | M2 | 484 | 5 | 3 | 482-486 | 0.161 | 0.000 | 5 | 476-490 | -0.052 | 0.000 | KF22632 |
| HdIV8 | F: FAM-ACGTGATGGATGTCAGTACG <br> R: AGTTACCCTATGACTCTGGCA | $(\mathrm{GT})_{5} \mathrm{AT}(\mathrm{GT})_{8}$ | M1 | 333 | 11 | 8 | 329-347 | 0.080 | 0.000 | 10 | 329-353 | 0.037 | 0.001 | KF22633 |
| HdIV9 | F: PET-GGGACCCAAATGATCAACAG <br> R: GCCTCAACTGCTGCCAATTA | $(\mathrm{GT})_{9}$ | M1 | 126 | 5 | 3 | 122-126 | -0.045 | 0.000 | 5 | 122-130 | -0.068 | 0.000 | KF22634 |

[^1]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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[^1]:    Freq $N A$ frequency of null alleles

