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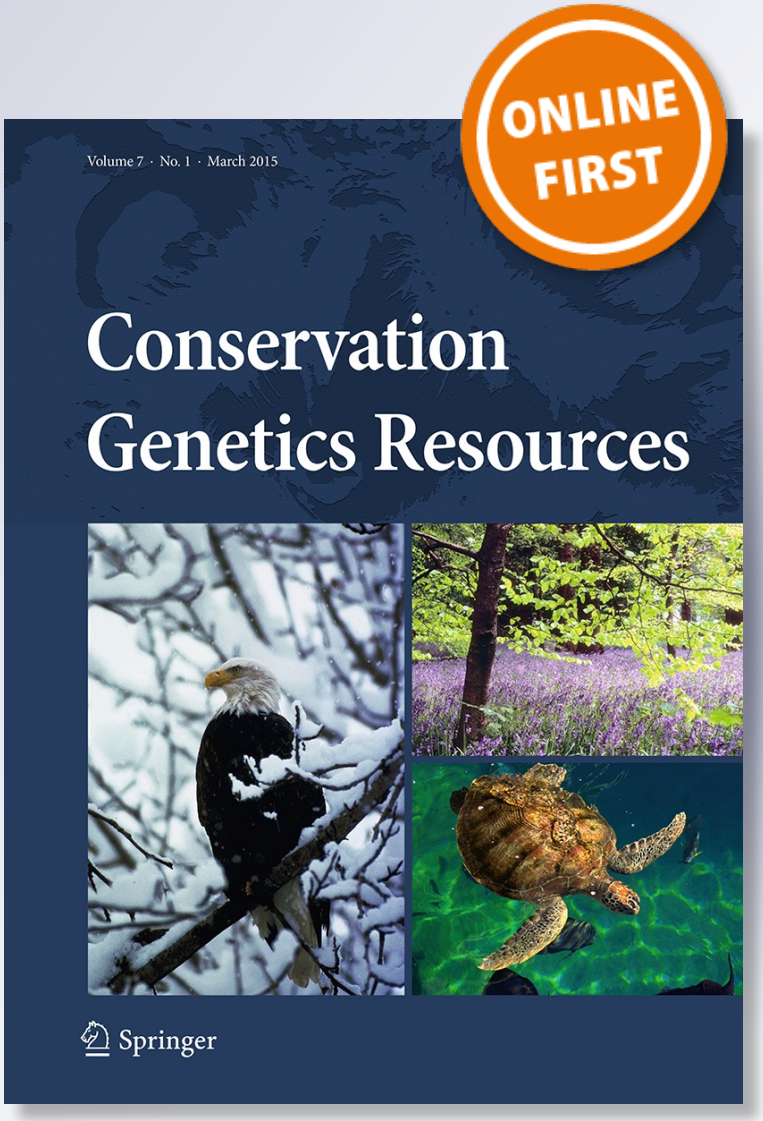

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MICROSATELLITE LETTERS

Microsatellites for the parasitoid wasp *Hyposoter horticola*

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Abstract We developed 14 microsatellite loci for the solitary parasitoid wasp *Hyposoter horticola*, an egg-larval endoparasitoid specialist of the Glanville fritillary butterfly *Melitaea cinxia*, in the Åland islands, Finland. The microsatellites were developed from a 454 sequencing enriched library. The characteristics of the primer sets and polymerase chain reactions for these microsatellite markers are presented. Thirteen of the 14 loci tested were polymorphic with on average 3.6 alleles per locus (range 3–6). The expected (H_E) and observed (H_O) heterozygosities ranged from 0.19 to 0.76 and 0.20 to 0.62 respectively. These markers can be used to study population genetics and genetic relatedness in *H. horticola* or related taxa, and to assess the presence of the parasitoid in hosts.

Keywords Hymenoptera · *Hyposter horticola* · *Melitaea cinxia* · Microsatellites

Hyposoter horticola (Gravenhorst) (Ichneumonidae: Campoplaginae) is an egg-larval endoparasitoid, specialist of the Glanville fritillary butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae) (Shaw et al. 2009), which lives as a classical metapopulation in the Åland islands in Finland. The butterfly metapopulation persists in stochastic balance between local extinctions and colonisations, with around 500 of the nearly 4000 potential habitat patches occupied in any given year (Hanski 2011). The parasitoid wasp *H. horticola* is extremely dispersive and is present in almost all the local host populations, and in virtually all host nests a fraction (around one-third) of the caterpillars is parasitized (Montovan et al. 2015).

We developed 14 microsatellite markers in order to study population genetic structure, female dispersal range, genetic relatedness of offspring coming from the same host nest, and presence of *H. horticola* in a host caterpillar. These markers can potentially also be used for the study of related taxa, some of which, such as *H. didymator,* are important natural enemies of agricultural pests.

A microsatellite library was developed by the Cornell Life sciences Core Laboratory Center, using four female *H. horticola* and following protocols modified from Hamilton et al. (1999). We then aligned the obtained contigs using Geneious version 5.5 and designed primer sets for 14 loci using the software Primer3. The specific annealing temperature T_a was determined for each locus by performing a gradient PCR. The forward primer from each pair was labelled with a fluorescent dye (6-FAM, HEX or TAMRA). Each 10 μL polymerase chain reaction (PCR) contained 0.4 μM of F primer, 0.5 μM of R primer, 200 μM of

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Table 1 Characteristics of the *Hyposoter horticola* microsatellite loci: locus and NCBI Probe accession number, repeat motif, primer sequences (*F* forward, *R* reverse, [a] labelled primer), locus-specific annealing temperature (T_a) in °C, number of alleles (# alleles), size range of the alleles in base pairs (range), observed (H_O) and expected (H_E) heterozygosities

Locus Accession no	Repeat	Primer sequences (5′–3′)	T_a	# alleles	Range	H_O	H_E
Hho10	AC	F: AAAATGTACAGATGGCCCGT	55	1	244	–	–
Pr032301863		R: TGCTTGAATGAGGAGGTTGA[a]					
Hho11	AC	F: CCGCAGAGCTATTACTCCCTT[a]	55	3	189–199	0.20	0.36
Pr031826694		R: GAGGAGACACGGTTCGAAAG					
Hho12	AC	F: TTCCACTCCATGGATTCTGC[a]	57	3	151–159	0.40	0.41
Pr031826695		R: TCGCTCTCCACTCTTCGTTT					
Hho13	AAG	F: TCGCCTACTCATGTACTCCTCGTT[a]	59	3	244–253	0.53	0.59
Pr031826696		R: TATACCCAGGTCTTTCTTCCGCCT					
Hho15	AC	F: ATGCCGCCTATGCAACGGAAA[a]	57	3	102–110	0.25	0.3
Pr031826697		R: ATATTTCGCAAGCCCGTGTGC					
Hho16	AC	F: TCTGGGTGCATCGCTCAATA[a]	59	5	215–232	0.49	0.56
Pr031826698		R: TCACGCGAATCCGATGAATTG					
Hho17	AC	F: CGCTATGCTTTGTCTTGCCT[a]	55	6	263–273	0.62	0.76
Pr031826699		R: ACGATTAATTTTCGGCGGTT					
Hho18	AC	F:CGCACTTCTCTTGTACTAACACAC[a]	55	3	126–150	0.2	0.19
Pr031826700		R: GCATTCGAACGCTGTGTACT					
Hho19	AG	F: GTTTGAGGGAGAAACGCTGA[a]	54	4	270–275	0.58	0.61
Pr031826701		R: GCATCTCTATCCTGTGTCCCA					
Hho21	AG	F: AGTTTCTGACTTGCCTGCCT[a]	56	3	109–113	0.51	0.59
Pr031826702		R: CTGGTGGATTTGCTCGATTTGC					
Hho22	TC+AC	F: GACGAGACGGACGTGGTAAG[a]	59	5	294–306	0.45	0.52
Pr031826703		R: AATTTCGAATTGAGGCGGAT					
Hho23	AG	F: ATCCCGGCATTAAGTTCAGA[a]	56	3	142–146	0.24	0.27
Pr031826704		R:TTTAGGAGATTATTGCTCCACTATG					
Hho24	AG	F:GAGCAGAGAGACACAAGGTGAA[a]	56	3	158–166	0.54	0.63
Pr031826705		R: CGTCAAGCATCGAACAAAAA					
Hho25	AG	F: TATCGCGACGATCTTGCTGT[a]	54	3	298–306	0.27	0.35
Pr031826706		R: TGTGCATACGCTCTCTCGAA					

DNTPs, 1 μL of buffer, 0.2 U of dyNAzyme Taq DNA polymerase and 1 μL of DNA extract. All amplifications were conducted with Bio-Rad S1000 thermal cyclers using a denaturation phase at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, locus specific T_a for 30 s and 72 °C for 1 min, and ending with 72 °C for 5 min and 4 °C for 15 min. Loci were amplified in six multiplexed PCR reactions, and the diluted PCR products were run in three panels using an ABI 3730 automated sequencer. Genotypes were scored using GeneMapper version 4.1 (Applied Biosystems).

We used the software Micro-Checker to test for the presence of null alleles and allele dropouts, and Excel Microsatellite Toolkit version 3.3.1 to estimate Nei's unbiased gene diversity (H_E), observed heterozygosity (H_O), and number of alleles for each microsatellite locus. Then we used Fstat 2.9.3.2. to test for linkage disequilibrium and Hardy–Weinberg equilibrium. Characteristics of the microsatellite loci developed are shown in Table 1. The observed number of alleles in a sample of 407 individual wasps collected from the Åland islands ranged from 1 to 6 and the mean observed (H_O) and expected (H_E) heterozygosities were 0.41 and 0.47 respectively. We did not detect any null alleles or allele dropouts in the data, and there was no linkage disequilibrium between pairs of loci. All loci were in Hardy–Weinberg equilibrium at the level of collection site (habitat patch), but there was a heterozygote deficiency at the whole population level.

Until now only two microsatellite loci specific to *H. horticola* were available (Kankare et al. 2004). This new set of markers will be valuable to study population genetics and offspring genetic relatedness.

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