Molecular Ecology Notes (2004) **4**, 231–233 doi: 10.1111/j.1471-8286.2004.00626.x

PRIMER NOTE

Characterization of microsatellite loci in two primary parasitoids of the butterfly *Melitaea cinxia*, *Cotesia melitaearum* and *Hyposoter horticola* (Hymenoptera)

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Abstract

Five polymorphic microsatellite loci were isolated from *Cotesia melitaearum* (Wilkinson) and three from *Hyposoter horticola* (Gravenhost). Both of these parasitic Hymenoptera use the butterfly *Melitaea cinxia* (Linnaeus) exclusively as their host in the Åland islands in SW Finland. Allelic diversity and heterozygosity were quantified in samples from the Åland islands and other localities around Europe. In addition, microsatellite loci for *C. melitaearum* were tested in five other *Cotesia* species: *C. acuminata* (Reinhard), *C. bignellii* (Marshall), *C. congregata* (Say), *C. cynthiae* (Nixon) and *C. koebelei* (Riley) from several different host species in Europe and Asia.

Keywords: *Cotesia*, cross-reactivity, *Hyposoter*, microsatellites, parasitoids, population structure

Received 20 December 2003; revision received 23 January 2004; accepted 23 January 2004

Parasitoids are abundant and diverse insects in nearly all terrestial ecosystems (Godfray 1994). By understanding the patterns of genetic variability within and among parasitoid populations and species we can interpret our growing knowledge of their behaviour, natural history and population dynamics in a broad ecological and evolutionary context. *Cotesia melitaearum* (Wilkinson) (Braconidae: Microgastrinae) and *Hyposoter horticola* (Gravenhost) (Ichneumonidae: Campopleginae) are two larval parasitoids restricted to Melitaeine butterfly hosts in Europe. In the Åland islands of SW Finland both parasitoids are almost entirely specialized on *Melitaea cinxia* (L.) (Lepidoptera: Nymphalidae) (Lei *et al*. 1997; van Nouhuys & Hanski 2004), which lives in small populations forming a classical metapopulation with a high rate of population turnover (Hanski 1999). The population ecology and biology of the parasitoids of *M. cinxia* has been intensively studied for 10 years in the Åland islands (Lei *et al*. 1997; van Nouhuys & Hanski 2004). Microsatellite markers were developed to study the spatial genetic structure and kinship of these parasitoids in relation to the metapopulation dynamics of their common host.

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Genomic DNA was extracted from five pooled *C. melitaearum* wasps and from one *H. horticola* wasp using standard phenol/chloroform extraction protocol described in Saccheri & Bruford (1993). A size-selected library (400–1000 bp) was constructed and transformant colonies were screened using digoxygenin labelled oligonucleotide probes (e.g. CT_{15}, GT_{15}, $GGAT_7$, $GGAA_7$, $GACA_7$) using a standard protocols (A. Estoup and J. Turgeon, pers. comm. Protocols available at: http://www.inapg.inra.fr/dsa/microsat/microsat.htm). One primer from each pair of primers was end-labelled with fluorescent dye (6-FAM, HEX, NED). Each 10 μL polymerase chain reaction (PCR) contained different concentrations of forward and reverse primers, 200 μM of each of the dNTPs, 1.5 mM $MgCl_2$, 20 ng of BSA and 0.5 U of Ampli Taq DNA polymerase. All amplifications were conducted with PTC 100 thermal cycler (MJ Research) using a denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, locus specific T_a for 30 s and 72 °C for 45 s. The Excel Microsatellite Toolkit (available at http://acer.gen.tcd.ie/~sdepark/ms-toolkit/) was used to estimate Nei's (1987) unbiased gene diversity (H_E), observed heterozygotes (H_O) and number of alleles (A) for each microsatellite locus (Table 1). Deviation from Hardy–Weinberg equilibrium was assessed by F_{IS} with FSTAT

Table 1 Characteristics of the *Cotesia melitaearum* and *Hyposoter horticola* microsatellite loci: number of individuals (females) scored, repeat motif, size of the cloned allele, number of alleles, primer sequences (* = labelled primer), primer concentration in PCR reaction, locus-specific annealing temperature, observed and expected heterozygosity and GenBank accession numbers

Locus	Repeat	Size (bp)	No of alleles	Primer sequences (5′–3′) (F = forward, R = reverse)	Primer conc. (pmol/μL)	T_a (°C)	H_O	H_E	GenBank No.
Cotesia melitaearum N = 151 (68)									
Cme1	$(GT)_9AT(GT)_5$	166	3	F: GAAGCGCTTTTGAATGAC	1.0	57	0.19	0.22	AB128899
				R: GTTTTGACGCCTTAGAAATC*	0.5				AB128900
Cme3	$(CA)_{11}$	142	1	F: CGAATGGCACAAACCTCAATA*	0.2	57	—	—	AB128901
				R: GAAGTTTCCCTGATGACCCTC	0.4				AB128902
Cme4	$(TG)_{23}T(TG)_6$	266	5	F: GTTTTCGACATGAAGGTCCCCTATC	0.2	59	0.39	0.70	AB128903
				R: GTGCCAGCAATGAGGATGA*	0.1				AB128904
Cme15	$(CA)_{22}$	102	15	F: GCAGATGATAGAAAGACTTAAGCT*	0.3	57	0.33	0.88	AB128905
				R: AGCTCTAATGTCACACAAGAAGAGA	0.6				AB128906
Cme17	$(AC)_3CC(AC)_6$	130	1	F: CGATGGTTTGAAGCCCTAAG	0.2	56	—	—	AB128907
				R: GATGAAATCGGTCAGGCTGT*	0.1				AB128908
Hyposoter horticola N = 217 (93)									
Hho1	$(GT)_9$	180	2	F: CGGAGTGCGTGCGGATTTTTT	1.0	59	0.09	0.12	AB128909
				R: TGAACAAAGTGATGCGGGCGG*	0.5				AB128910
Hho3	$(CA)_{46}$	138	12	F: GACTAACATCTGTGCAAAACGG	1.0	59	0.61	0.87	AB128911
				R: ACGCAATATCCCACAGCATCG*	0.5				AB128912
Hho5	$(CT)_{19}(C)_5(CT)_3$	248	8	F: GCAGCGTCGCAGCCTCAAAA	1.0	59	0.67	0.80	AB128913
				R: CTACCTCAATGTCTTCCAGCC*	0.5				AB128914

Table 2 Characteristics of the *Cotesia melitaearum* microsatellite loci examined in five species of *Cotesia* other than *C. melitaearum*, as well as *C. melitaearum* from *M. cinxia* from seven countries other than Finland and *C. melitaearum* from eight other host species than *M. cinxia* (pooled in *C. m.* column). N indicates the number of individuals scored; T_a the annealing temperature, SR the observed allele size range and A the observed number of alleles

Cotesia species (N) Locus	T_a (°C)	*C.a.* (78) SR	A	*C.b.* (29) SR	A	*C.c.* (6) SR	A	*C.c.** (4) SR	A	*C.m.* (217) SR	A	*C.k.* (5) SR	A
Cme1	57	NA	0	NA	0	NA	0	NA	0	148–206	19	NA	0
Cme3	57	NA	0	140–142	2	NA	0	NA	0	140–142	2	NA	0
Cme4	59	254–290	13	262–282	8	274	1	NA	0	256–326	25	NA	0
Cme15	57	98–108	3	98–108	4	134–136	2	66–70	2	86–130	22	94–112	3
Cme17	56	98–130	3	130–132	2	118	1	NA	0	260–320	8	NA	0

Cotesia species abbreviations are: *C.a.*: *Cotesia acuminata*, *C.b.*: *C. bignellii*, *C.c.*: *C. cynthiae*, *C.c.**: *C. congregata*, *C.m.*: *C. melitaearum*, *C.k.*: *C. koebelei*. NA = not amplified

version 2.9.3.2 (Goudet 2001). Because of the haplodiploid nature of inheritance of Hymenoptera such as *Cotesia* and *Hyposoter*, only data from females were used to calculate Nei's unbiased gene diversity and observed number of heterozygotes.

The polymorphism of the microsatellite loci was investigated in 25 *C. melitaearum* local populations or host patches (totalling 151 individuals) and in 127 *H. horticola* local populations (totalling 217 individuals) from the Åland islands. The polymorphism of the *C. melitaearum* microsatellite loci at a larger scale was further tested with *C. melitaearum* individuals reared from *M. cinxia* from seven countries other than Finland (Estonia, Sweden, UK, France, Spain, Russia and China) (Table 2). Moreover, the polymorphism was tested with *C. melitaearum* individuals reared from eight additional host species [*Euphydryas aurinia* (Rottemburg), *E. aurinia davidi* (Oberthür), *E.* desfontainii (Godart), *Melitaea athalia* (Rottemburg), *M. deione* (Geyer), *M. didyma*

(Esper), *M. parthenoides* (Keferstein) and *M. trivia* (Denis & Schiffermüller)] (Table 2). Finally, cross-species amplification of *C. melitaearum* microsatellites was tested with five additional *Cotesia* species [*Cotesia acuminata* (Reinhard), *C. bignellii* (Marshall), *C. cynthiae* (Nixon), *C. congregata* (Say) and *C. koebelei* (Riley)]. The polymorphism of the *H. horticola* microsatellites was tested with individuals from three additional countries (Sweden, Estonia, Spain).

Allelic diversity ranged from one to 15 alleles in *C. melitaearum* loci and from two to 12 alleles in *H. horticola* loci. Observed heterozygosities were lower than expected heterozygosities at all loci in both parasitoid species (Table 1), with the two most polymorphic loci showing a significant deviation from Hardy–Weinberg equilibrium, when all subpopulations are combined. However, when treated separately, most of the *Hyposoter* subpopulations did not show any deficit of heterozygotes, indicating that significant F_{IS} at the larger scale is due to spatial structure. Some of the *Cotesia* subpopulations did show a significant heterozygote deficit, which could be due to inbreeding in these particular subpopulations. Two of the *C. melitaearum* microsatellites were monomorphic in Åland (Table 1), but polymorphic when tested with *C. melitaearum* reared from *M. cinxia* from Europe and Asia (Table 2). Some of the loci worked well with other *Cotesia* species (Table 2), broadening the scope of their applicability and expanding the library of markers now available for this genus (Jensen *et al*. 2002). All three microsatellite loci isolated from *H. horticola* were polymorphic in Åland, with unique alleles within regions at a larger geographical scale. To our knowledge these are the first published microsatellite primers developed from *Hyposoter* and might therefore be tested with some other *Hyposoter* species, including those important in biocontrol.

Acknowledgements

We warmly thank the numerous collectors who provided or identified the samples used in the study: R.R. Askew, G.N. Burton, P.W. Cribb, H. Decimon, C.U. Eliasson, L. Guanchung, I. Hanski, J. Jantunen, A. Komonen, G. Néve, U. Norberg, J. Pateman, J. Planas, D.L.J. Quicke, P.J.C. Russell, M.R. Shaw, M. Singer, L. Slaughter, C. Stefanescu, P. Summers, R. Sutton, S. van Nouhuys, V. Vikberg, B. Wee, N. Wahlberg and J. B. Whitfield. We sincerely thank Toshka Nyman for help in the laboratory. Comments from Saskya van Nouhuys helped to improve an earlier version of the manuscript. The study has been financially supported by Academy of Finland Grant nos. 38604 and 44887 to Ilkka Hanski, Finnish Centre of Excellence Programme (2000–05).

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