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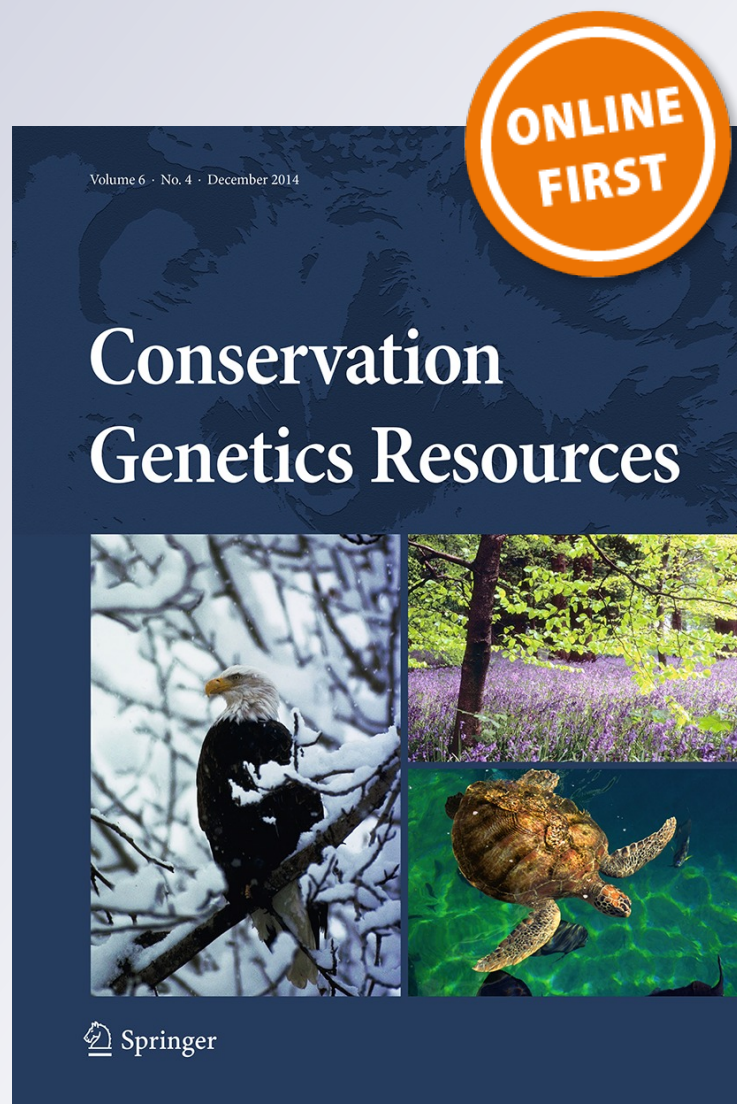
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Volume 6 · No. 4 · December 2014

Conservation Genetics Resources

Springer

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MICROSATELLITE LETTERS

Microsatellite markers for a hyperparasitoid wasp from a fragmented landscape

Abhilash Nair · Saskya van Nouhuys

Abstract We developed 31 microsatellite loci for a specialist hyperparasitoid wasp, *Mesochorus cf. stigmaticus* that parasitizes the parasitoid *Hyposoter horticola*, in Åland islands of southwestern Finland. The microsatellites were developed from 454 sequencing of the enriched libraries. The details of primers and the polymerase chain reactions for these microsatellite markers are described. All the loci tested on 24 samples were polymorphic with on average 4.1 alleles per locus (range 2–13). Genetic diversities as measured by average expected heterozygosity (H_E) and allelic richness (A_R) were generally high ($H_E \approx 0.10$–0.80; $A_R \approx 1.9$–11.5). These markers will be useful in population genetic studies of this parasitoid and other related taxa.

Introduction

Mesochorus cf. stigmaticus (Ichneumonidae: Mesochorinae) is a hyperparasitoid that primarily lays eggs into the larva of the parasitoid *Hyposoter horticola* (Ichneumonidae:

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Campopleginae) within the caterpillar of the Glanville fritillary butterfly *Melitaea cinxia* (Shaw et al 2009). The butterfly lives as a classical metapopulation in the Åland archipelago in southwestern Finland. These islands have about 4,000 habitat patches in a 50–70 km area, of which the butterfly occupies about 300–500 habitat patches in a given year (Hanski 2011). The parasitoid *Hyposoter horticola* is a solitary egg-larval endoparasitoid. It is extremely mobile and parasitizes a third of butterfly caterpillars throughout the landscape every year (Montvan et al. 2015). The solitary hyperparasitoid *Mesochorus cf. stigmaticus* lays eggs into the larvae of parasitoid *Hyposoter horticola*. It is also highly dispersive and is found in most of the local butterfly populations (van Nouhuys and Hanski 2005). Members of the genus *Mesochorus*, which are all hyperparasitoids, are generally considered as specialists with respect to host Lepidoptera species, parasitizing one or more species of parasitoids within the host caterpillars. The taxonomy of the genus *Mesochorus* is not well resolved but the species present in Åland islands is probably the same as the European species *Mesochorus stigmaticus* Brischke, 1880; and has been reared from *H. horticola*, and occasionally from *Cotesia melitaearum* (Braconidae) developing within *M. cinxia* caterpillars (Shaw et al 2009).

We developed 31 new polymorphic microsatellite markers, which can be used to study the dispersal and population genetic structure of this species, and can also be useful in resolving the taxonomic uncertainties within this genus. Further, these markers may be of use in programs on biological control because several *Mesochorus* species are known to disrupt biological control in agriculture.

A microsatellite library was developed using protocol modified from Hamilton et al. 1999, and sequenced using 454 sequencing facility at the Cornell Life sciences Core Laboratory Center. Sequences having microsatellite repeats

Table 1 Microsatellite loci for *Mesochorus cf. stigmaticus*. *Ta* Annealing temperature, *N* number of individuals, observed (H_O) and expected (H_E) heterozygosity, *Ar* allelic richness, *H–W p* value for deviation from Hardy–Weinberg equilibrium

GenBank	Loci	Sequence	Label	Repeat	Ta	N	A	Range	Ar	H_O	H_E	H–W
KP315922	Meso_1072F Meso_1072R	5′ TAG CAG TTT GAC GTT GTC CTT C 3′ 5′ GGC TTG ACT GAG AAA GTG TCC T 3′	HEX	(CT)11	55	22	3	175–199	2.818	0.227	0.281	0.4253
KP315929	Meso_128F Meso_128R	5′ CGA AAC GCG AAT CGA GAC AC 3′ 5′ TGG AAG CCG AGG ACC ATC TA 3′	TAMRA	(AG)13	55	22	5	201–229	4.941	0.636	0.685	0.2387
KP315931	Meso_1293F Meso_1293R	5′ TGT ATA CCG GCG CCA ATG TT 3′ 5′ AGT TTA TCA ACG CGC GTG AC 3′	FAM	(GT)12	55	20	6	231–261	5.900	0.550	0.796	0.0044
KP315932	Meso_1488F Meso_1488R	5′ CAC ACT CAA AAC AAT AAT CCG C 3′ 5′ CAC AGA CGT ACA GGG ACT TTC A 3′	TAMRA	(AAC)5	55	21	3	303–327	2.983	0.381	0.390	1.0000
KP315919	Meso_151F Meso_151R	5′ CAG AAA GAT AAC GCC CCG GA 3′ 5′ AGC TCC TTG TAC CGT TAC GC 3′	HEX	(CGAT)6	55	23	5	169–209	4.738	0.522	0.440	1.0000
KP315933	Meso_1639F Meso_1639R	5′ CGA ACC AGT TTT CGT GCC 3′ 5′ CTT TTC TGC TAC CGT GAT ACC C 3′	FAM	(AGT)6	55	24	4	156–198	4.000	0.750	0.700	0.1480
KP315923	Meso_1743F Meso_1743R	5′ GAC AAG AGG TTT ACG CGG AT 3′ 5′ CGG TCC TTC GCT ATA CAG TTT C 3′	TAMRA	(AG)12	55	23	6	167–201	5.946	0.565	0.735	0.0033
KP315924	Meso_1875F Meso_1875R	5′ GTC TCG TCG CTG GTG GTT TAT 3′ 5′ GGA GAA GAT TAC TCG AAT TTG TGC 3′	FAM	(CT)10	55	24	7	170–214	6.441	0.625	0.761	0.2905
KP315941	Meso_2176F Meso_2176R	5′ GCG CCT GTT GCT GTT TT 3′ 5′ CTG CGG CGA TGT ACC AAA TG 3′	TAMRA	(GTT)5	57	22	2	195–215	1.970	0.000	0.091	0.0234
KP315925	Meso_2224F Meso_2224R	5′ AGA AAA CGG AAG TAA GGT GCA A 3′ 5′ TGA CGA AGA TAC GAG AAT CAG G 3′	HEX	(CT)12	55	22	5	255–283	4.607	0.455	0.531	0.3157
KP315926	Meso_2376F Meso_2376R	5′ TCC TTC CTT ACC GAC AAC AAG T 3′ 5′ TCC ATC GAC AAG ATT GAC CTT T 3′	TAMRA	(AC)12	55	24	13	228–278	11.534	0.500	0.745	0.0281
KP315918	Meso_24866F Meso_24866R	5′ GCA AAG GTC GAG CCA AGT 3′ 5′ GAT TTC TGC ATA TCT CGT CGC 3′	FAM	(AGAT)6	60	20	6	180–220	5.800	0.700	0.693	0.5210
KP315920	Meso_2684F Meso_2684R	5′ GCA TAC CGC CGA TGA ATC TG 3′ 5′ TGT AGA TTA ATG CTT ACG CCC C 3′	TAMRA	(GCGT)6	55	21	2	235–255	2.000	0.000	0.524	**0.0000**
KP315921	Meso_29292F Meso_29292R	5′ GTA ACG ATG AAG GAA AGT TCG G 3′ 5′ AGA GAG GAG GGA GCG TGA AT 3′	FAM	(ACTG)6	60	22	4	247–275	3.784	0.091	0.256	0.0050
KP315927	Meso_3121F Meso_3121R	5′ CCT GCT CCT TTT CGT CAA GTT A 3′ 5′ ACT CGG TTG ATT TTG CTT CAG T 3′	FAM	(TC)6	55	24	2	143–165	2.000	0.333	0.337	1.0000
KP315928	Meso_3466F Meso_3466R	5′ TGC GTT ACT GCT TCT TGC TAT T 3′ 5′ GAC TAC CCG CTT GGT GGT T 3′	HEX	(CT)12	55	24	5	142–170	4.441	0.500	0.583	0.1658
KP315934	Meso_3777F Meso_3777R	5′ CTC CAT CGA ATC ATA CCA GCT C 3′ 5′ TGT TCT TGC TGT CCT TGT TGT C 3′	HEX	(AAC)5	55	19	3	197–254	2.999	0.316	0.284	1.0000

Table 1 continued

GenBank	Loci	Sequence	Label	Repeat	Ta	N	A	Range	Ar	H_O	H_E	H–W
KP315945	Meso_4376F	5′ CGC TCG GGT CTA TAT CGT GT 3′	FAM	(AAG)6	55	24	3	163–189	3.000	0.625	0.630	0.9405
	Meso_4376R	5′ ATC GCG CAC CAC ATC CTT AT 3′										
KP315943	Meso_4466F	5′ AGC TCA CCG TGC AGT AAC AA 3′	TAMRA	(AAC)9	60	23	5	213–245	4.738	0.522	0.641	0.3526
	Meso_4466R	5′ ATT GCC GTT CGA GTT TGT GC 3′										
KP315935	Meso_4776F	5′ ACG ATC TCT CTT CCG TTT CTT C 3′	TAMRA	(AAG)8	55	24	5	151–181	4.691	0.458	0.548	0.0742
	Meso_4776R	5′ CGT ATT CGT ATT CCG GTT CTT C 3′										
KP315936	Meso_6047F	5′ TCT AAT TCG TCC TTC CCT TTC A 3′	FAM	(AAC)5	55	23	2	331–353	1.992	0.130	0.125	1.0000
	Meso_6047R	5′ ATA AAA TCC GTG TTG AGG ATG C 3′										
KP315917	Meso_6407F	5′ CGC AAC CAC ATA TCT CAA CAA T 3′	TAMRA	(ATCT)6	55	20	5	176–200	2.000	0.100	0.266	0.0312
	Meso_6407R	5′ CTC TCA AGT GAT GCG CTT ACC 3′										
KP315930	Meso_647F	5′ TGT CCC TAG AGC GAC GAA GA 3′	HEX	(CT)11	55	18	5	308–336	5.000	0.611	0.735	0.5656
	Meso_647R	5′ AGA AAA GTT TGA GCA TAT CCA GTC G 3′										
KP315937	Meso_6811F	5′ CAG CAT AGT CCA GTA CGA GCA C 3′	HEX	(AAC)9	55	21	3	150–176	2.857	0.095	0.330	0.0019
	Meso_6811R	5′ GAC TTC CTC CAT GAA TCT TTG C 3′										
KP315938	Meso_7542F	5′ ACA ACT CGT TTT CGT AAT CCG T 3′	TAMRA	(AAC)12	55	20	4	373–401	3.999	0.550	0.599	0.9367
	Meso_7542R	5′ AAT TTG TCT GGC TTC TTT CGA G 3′										
KP315939	Meso_7718F	5′ ACG ACC GAG AGA TAC AAT GAA A 3′	FAM	(AAC)9	55	22	3	287–313	2.818	0.136	0.511	**0.0002**
	Meso_7718R	5′ TAC GAA TTT AAC CAA CGT CCA C 3′										
KP315944	Meso_8416F	5′ GGC GCC GAT TTG ATC TTC AC 3′	HEX	(CTT)7	55	21	3	312–338	3.000	0.381	0.505	0.1462
	Meso_8416R	5′ CAT TGC TGG CTT GAC TGC TG 3′										
KP315940	Meso_8726F	5′ TGA ACT CGT CGT CTC CAA TTT A 3′	HEX	(AAG)8	55	24	3	185–211	3.000	0.542	0.673	0.3242
	Meso_8726R	5′ TCT CAG CAG CAC GTA GGT TTT A 3′										
KP315942	Meso_9582F	5′ TTA AAC GCC GAG GCT TCT GT 3′	FAM	(GGT)8	59	21	2	141–164	2.000	0.429	0.343	0.5363
	Meso_9582R	5′ AGC GTC ATC ATC AGC CTG TT 3′										
KP315916	Meso_2630F	5′ TGC CTT CAT CGA TCG GCT TT 3′	HEX	(ATCT)7	55	24	3	177–214	2.750	0.250	0.291	0.5076
	Meso_2630R	5′ GGA GCC AGA GTC CTA GTT GC 3′										
KP315915	Meso_5041F	5′ TCC GTC ATA CAG TCG GTT GC 3′	FAM	(ATCT)10	60	20	3	307–331	3.000	0.350	0.413	0.3507
	Meso_5041R	5′ TGC ACT ACG GCG AAA GTG AT 3′										

Bold values for H–W indicate loci that have possibility of null alleles

and flanking regions for designing the primers were identified using program TANDEM REPEAT FINDER. Initially, 550 unique microsatellites sequences were identified, from which primers were designed for 31 sequences using program OLIGO 7. These primers were tested on 24 individuals using forward primers labeled with a fluorescent dye (FAM, HEX or TAMRA). The PCR reactions were performed in a total reaction volume of 10 µl that contained $1\times$ QIAGEN Multiplex PCR solution, Q solution, 0.2 µM of each primer, dH$_2$O and 10–20 ng of template DNA. The PCR amplification conditions were as follows: 95 °C for 15 min, followed by 30 cycles of 95 °C for 30 s, 55–60 °C for 1.5 min, 72 °C for 1 min with a final step at 60 °C for 10 min. The diluted PCR products were electrophoresed on an ABI 3730 automated sequencer and genotypes were scored with GeneMapper version 4.1 (Applied Biosystems). Basic polymorphism indices (Allelic richness, expected and observed heterozygosity) were calculated with programs FSTAT 2.9.3 and ARLEQUIN 3.5.1.2. Tests for deviations from the Hardy–Weinberg equilibrium and linkage disequilibrium were done using the software GENEPOP ver 4.2.

The locus specific results are summarized in Table 1. The number of alleles ranged from 2 to 13 with a mean of 4.10 alleles per locus. The mean observed heterozygosity (H_O) and expected heterozygosity (H_E) were 0.398 and 0.498 respectively. There was no linkage disequilibrium between any pair of loci, but two loci deviated from Hardy–Weinberg expectations after bonferroni correction (Table 1). MICROCHECKER analysis indicated that these loci also showed significant heterozygote deficiency and might have presence of null alleles (Table 1). These microsatellite loci are the first genetic markers developed for the parasitoid genus *Mesochorus* and should be a valuable resource for ecological studies of this lesser known species.

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