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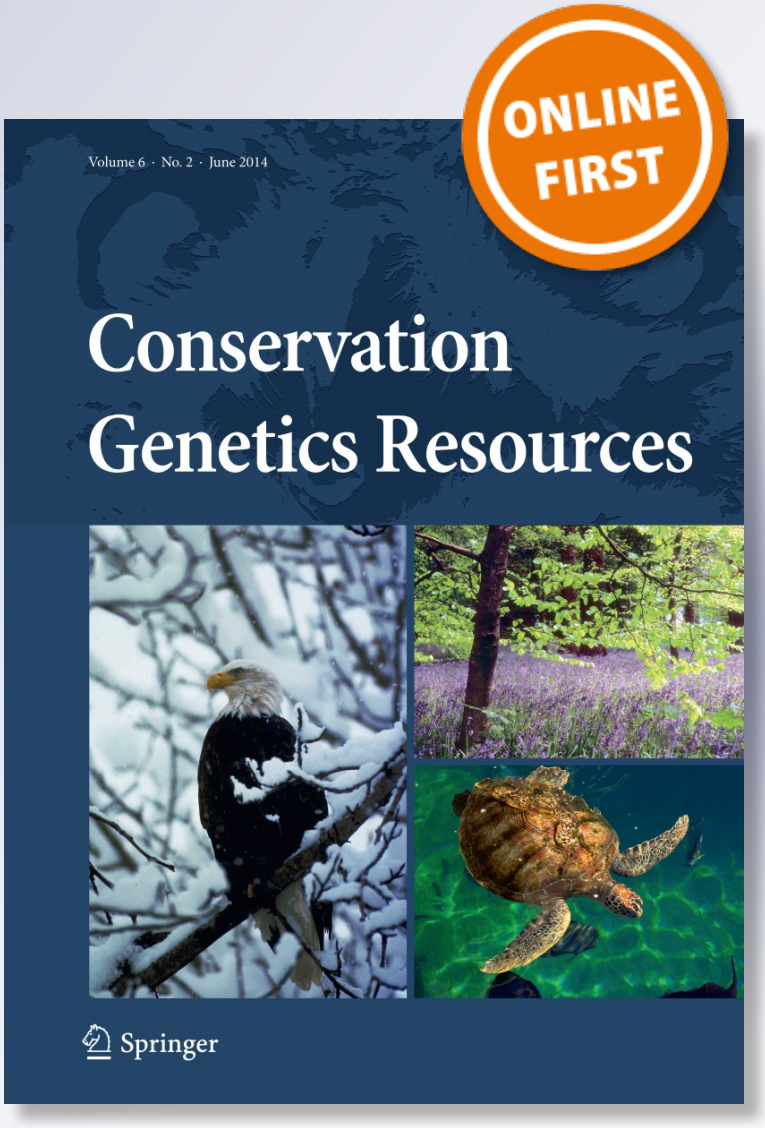

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MICROSATELLITE LETTERS

The development and characterization of polymorphic microsatellite loci for the genus *Melinaea* (Nymphalidae, Ithomiini)

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Abstract Due to their preference for undisturbed habitats, the butterflies of the genus *Melinaea* are promising indicators of ecological conditions. Here we describe 12 polymorphic microsatellite markers, with 3–26 alleles per locus, an observed heterozygosity of 0.138–0.889, and an expected heterozygosity of 0.400–0.970. These markers will prove useful in investigating patterns of differentiation in this clade.

Keywords Aposematic · Diversification · Lepidoptera · Mimicry · Speciation

The tribe Ithomiini (Nymphalidae: Danainae) represents a large group of Neotropical butterflies which numerically dominate forest communities. They prefer undisturbed forests, and have been proposed as promising biological indicators of ecological conditions and biological diversity. The species of the genus *Melinaea* are some of the largest ithomiines, but are difficult to separate from one another as a result of mimicry and polymorphism, and mitochondrial markers have failed to separate the different species (Whinnett et al. 2005). Here we present primers that amplify 12 microsatellite loci for three closely related *Melinaea* species.

Microsatellites were developed using 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries (Malausa et al. 2011) by GenoScreen (http://genoscreen.fr). Primers for candidate microsatellite loci were designed with the aid of Primer3 v.0.4.0 (Untergasser et al. 2012).

DNA was extracted from thoracic tissue using Qiagen DNeasy tissue kits. PCR conditions (12.5 µl volume) were: 1 µl of 10× Taq Buffer, 0.2 mM dNTPs, 0.2 U *Taq* Polymerase (Qiagen), 5–40 ng DNA, 0.1 µM M13-tailed forward primer, 0.4 µM dye-labelled (NED, VIC, PET and 6-FAM) M13 primer (5′CACGACGTTGTAAAACGA C3′), and 0.4 µM reverse primer. The PCR amplification temperature profile consisted of an initial denaturation at 92 °C for 2 min, 45 cycles at 92 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, and a final elongation at 72 °C for 15 min (Table 1).

PCR products were multiplexed (up to 4 loci) and 1 µl of the resulting solution was added to 8.8 µl Hi-Di formamide (Life Technologies) and 0.2 µl size standard (Life technologies Genescan 500LIZ). Products were resolved on an ABI 3130 Genetic Analyzer capillary DNA sequencer and scored and binned using SoftGenetics GeneMarker v.2.4.0. Amplification success and polymorphism of the reported microsatellites and of 1 microsatellite developed for *Heliconius numata* (Chouteau et al. 2014) were tested on *M. marsaeus phasiana*, *M. menophilus ssp* n 1, and *M. satevis cydon*, from Tarapoto, Peru (Table 2).

Twelve polymorphic loci were found and the number of alleles ranged from 3 to 26 per locus. Observed and expected heterozygosities and estimates of linkage disequilibrium between loci were calculated using GenePop

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Table 1 Microsatellite markers developed for *Melinaea*

Locus	Repeat motif	Primer sequences (5′–3′)	Fragment length (bp)	Genbank accession nb
mel3	TG	F: CGGCACATTCTGGTTGTA	117–188	KJ908169
		R: CGAGTTCCTATACATCAATTTT		
mel4	CA	F: AACCATTTCACCAACCATTC	205–375	KJ908170
		R: AGCAGTGTCTGTGTGCTTGT		
mel9b	CAA	F: TTTTGTGTATCTTTAAGCAATGG	133–401	KJ908171
		R: GGATTCAACAGAATGGCAGT		
mel16b	TG	F: TATGACTGGTTGCTGGGTAA	182–300	KJ908172
		R: GCTGTTCCAGTGAAGTCAGA		
mel18	CA	F: AAAGCTTTATAAATATCGCATGT	129–220	KJ908173
		R: GCAGACGTGAAGACAACTGA		
mel27	TTC	F: ATCGGCTTATGTGAGGTGTT	168–277	KJ908174
		R: AAGCCTATACCTTGCAGTGG		
mel29	CATA	F: AGCAATACACGATTTGCTGT	102–297	KJ908175
		R: TGAGCAGACGTACTCGTCAT		
mel37	TGGA	F: ATTGTTCCACTGCTTTTCACC	250–279	KJ908176
		R: AGAATACGCAAAGTCGGCAG		
mel38	TGGATG	F: AGTCCGTAATTGGCACATCA	129–209	KJ908177
		R: TCGTGTGAACTGAGGAATCG		
mel42	ACAT	F: CCACGCAACAGGACTGAAG	148–220	KJ908178
		R: TGAGTACTGTATGGAAGAGACGTG		
mel43	ATAGG	F: CCGAAGATCAATAAGAAGGATCA	117–169	KJ930187

Table 2 Characterization of microsatellite loci for 3 *Melinaea* species, including the number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e). Loci marked with 1 belong to Chouteau et al. Loci for which observed heterozygosity deviates from expectations under HWE are marked with * for p value <0.01

Locus	*M. marsaeus phasiana* (n = 39)			*M. menophilus ssp n 1* (n = 37)			*M. satevis cydon* (n = 19)		
	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
mel3	15	0.400*	0.870	21	0.714*	0.940	15	0.588*	0.907
mel4	26	0.382*	0.941	26	0.394*	0.939	24	0.632*	0.970
mel9	12	0.138*	0.903	19	0.583*	0.871	13	0.177*	0.902
mel16	21	0.222*	0.937	20	0.250*	0.903	15	0.556*	0.925
num17-1[1]	22	0.824	0.832	18	0.828	0.916	18	0.889	0.940
mel18	14	0.606	0.885	15	0.865	0.890	10	0.706	0.879
mel27	15	0.641	0.825	21	0.800	0.940	12	0.778	0.908
mel29	17	0.700	0.927	16	0.667	0.828	15	0.833	0.897
mel37	12	0.658	0.810	7	0.694	0.711	9	0.632	0.861
mel38	11	0.588	0.831	7	0.531	0.702	7	0.500	0.835
mel42	6	0.378	0.417	14	0.622	0.743	7	0.611	0.698
mel43	8	0.615	0.621	5	0.611	0.719	3	0.421	0.400

v.4.2 (Raymond and Rousset 1995). Observed heterozygosity (H_o) ranged from 0.138 to 0.889 and the expected heterozygosity (H_e) ranged from 0.400 to 0.970. Four loci displayed significant deviation from Hardy–Weinberg equilibrium (HWE) (p value <0.01) caused by heterozygote deficiency, and may indicate the presence of null-alleles.

The obtained set of 12 microsatellite loci will prove invaluable to investigate patterns of differentiation and speciation in this clade of rapidly diversifying species.

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