

Microsatellites for the at-risk Mottled Duskywing butterfly, *Erynnis martialis*

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Method Article

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Abstract

We describe 24 microsatellite loci for an at-risk North American butterfly, the Mottled Duskywing (*Erynnis martialis*). Forty-one perfect tetra and di-nucleotide loci were originally identified using Illumina sequencing and were refined to 24 variable and cleanly amplifiable loci, which we attempted to characterize in 34 individuals from a single population. We detected 2 to 10 alleles per locus. Observed heterozygosity ranged from 0.06 to 0.76, and expected heterozygosity ranged from 0.06 to 0.79. We also describe conditions by which 18 microsatellites can be multiplexed in five separate reactions. These microsatellites will be used to investigate population differentiation and diversity of Mottled Duskywing, to inform reintroduction and conservation protocols for the species in Ontario, Canada where it is listed as endangered.

Full Text

The Mottled Duskywing (*Erynnis martialis*; Scudder, 1870), is a butterfly of the skipper family (Lepidoptera: Hesperiiidae; Pyrginae), endemic to North America and listed as endangered in Canada (COSEWIC 2015). We developed microsatellites to characterize genetic status of extant populations and inform planned reintroductions of the species.

Twelve female butterflies, collected from a single population in Marmora, Ontario, Canada, were transferred to the Cambridge Butterfly Conservatory, Ontario, Canada, for a captive rearing program. After they had died, genomic DNA was extracted from the head, thorax, and legs using a DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD). An equimolar DNA pool from all 12 individuals (105.84 μ L total) was sent to GenoScreen Services (Lille, France; www.genoscreen.fr), where a microsatellite-enriched genomic library was developed and sequenced using the Illumina MiSeq platform to generate 250 bp paired-end reads, following manufacturer guidelines from the MiSeq Reagent Nano Kit v2. Approximately 1.30 million reads were obtained and merged into 590,832 contigs with PrinSeq software. The program QDD v3 (Megléczy et al. 2014) was used to detect and select microsatellites and design primers. A total of 836 primer pairs were returned and of these, we tested 41 loci with perfect di- and tetra-nucleotide repeats for polymorphism and consistency in scoring.

Non-lethal wing tissue samples (Koscinski et al. 2011) were collected from 22 additional individuals from the same population in Marmora, Ontario, in the same year that whole females were sampled (2019), and DNA was extracted using a DNeasy Blood and Tissue kit. Genotypes from all 34 individuals were combined to characterize variation.

Each of the 41 selected microsatellites was initially amplified in three individuals in a 10 μ L PCR reaction containing forward and reverse primers (0.2 μ M), buffer (1X), $MgCl_2$ (3 mM), BSA (0.15 mg/ml), dNTPs (0.2 mM), AmpliTaq DNA polymerase (0.25 U), and 1 μ L DNA template. Thermal cycling parameters were: 96 °C for 5 min; 30 cycles of denaturation at 96 °C for 30 s, annealing at various temperatures for 30 s (Table S1), and elongation at 72 °C for 60 s; final extension at 72 °C for 5 min. Loci that displayed clear

bands of the expected size (based on sequencing results) on agarose gels were then amplified using one fluorescently labelled primer per pair, with optimized primer concentrations, and sized by fragment analysis on a 3730S capillary DNA analyzer, using LIZ-500 size standard (Applied Biosystems, Foster City, CA), at the University of British Columbia's Sequencing and Bioinformatics Consortium. Genotypes were called using GeneMarker® HID software (SoftGenetics, State College, PA). Loci that had more than two clear peaks, or could not be consistently called, were removed from further consideration. This left us with 24 loci that we attempted to characterize in the full set of 34 individuals (Table S1). We were able to amplify 18 of these loci in five separate multiplex reactions (Table S2).

Number of alleles per locus (N_a), observed and expected heterozygosity (H_o , H_e), and Hardy-Weinberg and linkage disequilibrium, were analyzed using genepop'007 (Rousset 2008).

Three loci (EMusat10, EMusat13, EMusat35) showed significant deviation ($P < 0.05$) from Hardy Weinberg equilibrium and evidence of null alleles, as assessed using MICROCHECKER v2.2.3 (Van Oosterhout et al., 2004; Table S1). The developed microsatellites have and will continue to inform conservation of Mottled Duskywing.

Declarations

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)
- [TableS2.xlsx](#)