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PRIMER NOTE

Highly polymorphic microsatellite loci for *Speyeria idalia* (Lepidoptera: Nymphalidae)

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Abstract

Four polymorphic microsatellite loci were identified in the butterfly *Speyeria idalia*. We constructed a phagemid library and screened approximately 120 000 inserts. Probing with GT15, we identified 36 positives and designed polymerase chain reaction (PCR) primers for 12 potential loci. Of those loci, only four consistently produced polymorphic, diallelic PCR products in the expected size range. These results are consistent with previous studies concerning the low frequency of microsatellite loci in the lepidoptera, although these four loci are highly polymorphic and therefore likely to provide information on the fine scale genetic structure among populations in this species.

Keywords: butterfly, Lepidoptera, microsatellite, Nymphalidae, primers, *Speyeria idalia*

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The identification of microsatellite loci in the lepidoptera has proven to be a difficult task (Nève & Meglecz 2000). Previous attempts to isolate polymorphic microsatellite loci have typically resulted in relatively few loci per species (e.g. Bogdanowicz *et al*. 1997; Meglecz & Solignac 1998; Keyghobadi *et al*. 1999; Reddy *et al*. 1999; Harper *et al*. 2000). We were interested in characterizing the genetic effects of recent habitat fragmentation in the butterfly *Speyeria idalia* (Lepidoptera: Nymphalidae). Previous studies utilized mitochondrial DNA (mtDNA) to detect population structure, but did not detect any restricted gene flow among recently isolated populations. We felt that microsatellite markers might be able to provide increased resolution for the detection of recently disrupted gene flow among those fragmented populations. Because microsatellites tend to be rare in the Lepidoptera, we chose to follow a method outlined by Hughes & DeLoach (1997), in which a phagemid library is constructed to allow for the rapid screening of over 100 000 inserts.

DNA was isolated using a sterile razor blade to homogenize a single thorax into a 'slurry' of tissue and incubated at 65 °C for 12 h in digestion buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, 20 µL dithiothreitol, 0.4 mg Proteinase K), followed by standard organic extraction procedures (Sambrook *et al*. 1989). Genomic DNA was digested with *Mbo*I and fragments between 300 and 1200 bases in size were excised from a 0.8% agarose gel with a sterile razor. Fragments were purified using Gene Clean (Bio101) following the manufacturer's recommendations and cloned into Lambda Zap Express (Stratagene) following the protocol outlined by Hughes & DeLoach (1997) with the following alterations. Recombinant phagemids were screened for GT repeats using ^{32}P-labelled GT_{15} oligos. Wash conditions were: 15 min in 5× SSC at room temperature, followed by 15 min in 2× SSC at room temperature, followed by two washes for 15 min in 2× SCC at 60 °C. We screened approximately 120 000 plaques and sequenced 36 positives. Primers were developed for 12 loci.

All the polymerase chain reactions (PCR) were optimized for each primer set in reactions containing 40 ng genomic DNA, 20 mM Tris-HCl, 50 mM KCl, 1, 2 or 3 mM $MgCl_2$, 0.25 mM of each dNTP, 5 µM each primer, 0.5 U Ampli-*Taq* Gold DNA polymerase (Perkin-Elmer), and water to a final volume of 20 µL. Each PCR optimization reaction was then subjected to an initial denaturation step at 94 °C for 12 min, followed by 35 cycles of amplification at 94 °C for 30 s, an annealing temperature ranging

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Table 1 Characteristics of four polymorphic microsatellite loci in *Speyeria idalia*. Annealing temperatures (T_a) are indicated for each locus and the optimal $MgCl_2$ concentration was 3 mM in all cases. Average expected (H_E) and observed (H_O) heterozygosities are based on a sample of approximately 25 individuals per population from 11 total populations found throughout the range of the species. GenBank accession numbers: AY039210–AY039213

Locus	Primer Sequence (5′–3′)	T_a (°C)	Repeat motif	Number of alleles	Size range	Mean H_O	Mean H_E
Si13	TAGTCAGCCAGTCATACGGT CGAGAACGATTTACAGAGAG	57	{CT(17)}CN{CT(4)}CCC{TG(7)}	46	247–339	18.3	22.5
Si17	CTAGCTAAACGTGAAGGAGT TACAACAACCTGCAGAGAGT	57	ACGC(21)	38	172–310	20.0	20.3
Si18	GTTACACCAAATCCACCAAG CATGTGCGTGGAATATGAGT	55	{CA(7)}T{AC(18)} {GC(7)}A{TG(4)}	76	139–313	17.8	21.6
Si31	CTCTCCGAGCAACTCTTCTA ATCTGTTTGAGAGCTGCGAT	57	{TGTC(4)}{TG(14)}	60	309–477	20.3	21.5

between 52 and 60 °C per reaction (in 2 °C increments) for 30 s, and 72 °C for 1 min. PCR products were amplified with one primer of each primer pair end-labelled with a fluorescent dye, either 6-FAM, HEX, or TAMRA, and then mixed with a size standard (Genescan-500 ROX) and run on an ABI 377. Genotypes were determined with GENOTYPER software (Perkin-Elmer).

Of the 12 loci for which primers were designed, one was monomorphic, seven produced alleles of unpredicted size or multiple alleles and four were polymorphic with reproducible, diallelic banding patterns in the predicted size range (Table 1). Approximately 25 individuals from 11 populations (275 individuals total) were genotyped to estimate the average observed and expected heterozygosities, which were calculated with the program GENEPOP (Raymond & Rousset 1995; Table 1).

The results of screening for loci in this butterfly species are similar to results reported in previous studies in that we were able to identify only four loci (Bogdanowicz *et al*. 1997; Meglecz & Solignac 1998; Keyghobadi *et al*. 1999; Harper *et al*. 2000; Nève & Meglecz 2000). However, these loci are unusual in that they are very highly polymorphic and will therefore be more likely to provide information about recent population structure where mtDNA has had little resolution.

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