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TECHNICAL NOTE

Development and characterization of twelve polymorphic microsatellite loci in the Bog Copper, *Lycaena epixanthe*

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Abstract We isolated and characterized 12 microsatellite loci from the Bog Copper, *Lycaena epixanthe*. Loci were screened in 24 individuals from one Maine location. Alleles per locus ranged from 3 to 12, observed heterozygosity ranged from 0.042 to 0.857, and the probability of identity values ranged from 0.032 to 0.780. These loci provide tools for examining the population genetics of Bog Coppers across a fragmented landscape.

Keywords Bog Copper · Butterfly · *Lycaena* · Microsatellite · PCR primers · SSR · STR

Bog Coppers (*Lycaena epixanthe*) occur in northeastern North America, extending south to West Virginia, west to Minnesota and Manitoba and north into the Maritime Provinces (NatureServe 2009). They are restricted to acid peat bogs. The lifecycle of the Bog Copper is intimately tied to the cranberry, *Vaccinium macrocarpon*, on which the adults and caterpillars feed (Cech and Tudor 2005; Wright 1983). Bog Coppers have a global conservation status ranking of G4. However, they are listed as imperiled in Pennsylvania and critically imperiled in West Virginia and Maryland (NatureServe 2009). The major threat to the species is the destruction of habitat; conservation of fragile acid bogs is essential for the survival of the Bog Copper (Cech and Tudor 2005).

We extracted total DNA from one individual *L. epixanthe*, using the DNeasy tissue kit protocol (Qiagen, Valencia, CA). We followed the enrichment procedure of Glenn and Schable (2005) with some exceptions. DNA was digested with restriction enzyme *Rsa*I (New England Biolabs), ligated to double-stranded linkers, denatured and hybridized to biotinylated microsatellite oligonucleotide mixes (mix 2 = $(AG)_{12}$, $(TG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$; mix 3 = $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACAG)_6$, $(ACCT)_6$, $(ACTC)_6$, $(ACTG)_6$; mix 4 = $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$), then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SimpleX-1 as a primer. There were two changes to the Glenn and Schable (2005) protocol. First, a new linker was used (SimpleX-1 Forward 5′-AAAACGTCGTGCGGA ATC and SimpleX-1 Reverse 5′-pGATTCCGCACGACG). Second, the enriched libraries were sequenced on a 454 FLX Genome Sequencer using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, Roche Company, Branford CT). Sequences were subjected to a 3′ quality trim where only one base in the last 25 bases of the sequence contains a quality score less than 20 or alternatively contains one ambiguous base. CAP3 [33] was then used to assemble sequences at 98% sequence identity using a minimal overlap of 75 bp. Along with singlets, contigs of two or three sequences were searched for the presence of microsatellite DNA loci using MSATCOMMANDER version 0.8.1 (Faircloth 2008) and primers designed with Primer3. One primer from each pair was modified on the 5′ end with an engineered

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sequence (CAG tag 5′-CAGTCGGGCGTCATCA-3′) to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals of *L. epixanthe*. PCR amplifications were performed in a 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04 μM tag labeled primer, 0.36 μM universal dye-labeled primer, 1.2 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Standard thermal cycling parameters consisted of 40 cycles of 96°C for 30 s, annealing temperature for 30 s, and 72°C for 30 s. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 65–55 or 60–50°C were used for some loci (Table 1). Touchdown cycling parameters consisted of 20 cycles of 96°C for 30 s, highest annealing temperature of 65°C (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 96°C for 30 s, 55°C for 30 s, and 72°C for 30 s. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard prepared as described in DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Twelve of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of 12 polymorphic loci in 24 specimens that came from one location, Harrington Heath in Harrington, Maine. Conditions and characteristics of the 12 loci are given in Table 1. We estimated number of alleles per locus (*k*), observed and expected heterozygosity (H_o and H_e), probability of identity (PI) using GenAlEx v6.0 (Peakall and Smouse 2006). Tests for deviations from Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction for multiple

Table 1 Details for 12 polymorphic microsatellite loci developed for *Lycaena epixanthe*

Locus	Primer Sequence 5′ → 3′	Repeat motif	Size (bp)	*N*	*k*	H_o	H_e	PI	T_a
Lyep1	F: TGTCATCGGAGGCCTATGTTT[a]	(GAT)^8	122–140	24	4	0.292	0.322	0.480	65
	R: AGGCGATGCCCTTCCAAAT								
Lyep4	F: TCTTGGCGTTCATTGAGGGA[a]	(GAT)^9	132–153	21	7	0.714	0.907	0.063	td65
	R: AGCTGGTATCTCGAAGGTTCT								
Lyep8	F: CGCTGTCGGTTGGATCTAGT	(ATC)^10	166–231	24	8	0.458	0.791	0.075	td60
	R: CGTCTTGGCGTTCTTTGAGG[a]								
Lyep9	F: CTCGGATTCTTAGCCCGTTT[a]	(ATCC)^14	155–215	24	12	0.458[b]	0.865	0.032	td60
	R: TTCGGGTTGCTTACATCTTT								
Lyep11	F: TGGCCGTATTGGATGCGAG	(GAT)^17	104–113	24	3	0.167[b]	0.351	0.450	td60
	R: CTTAGACTGCAAATAGCCGGA[a]								
Lyep15	F: ATTCAAGGGCTGCTGGAAGA	(AGAT)^9	202–230	24	7	0.500	0.760	0.092	td60
	R: TCTACTCGTGCTTTCTGTGT[a]								
Lyep18	F: GTTGGTCGGCCATTAACCAG[a]	(GAT)^11	227–249	23	7	0.739	0.692	0.150	td60
	R: CCTCAAGCAGAAGAATTTACCA								
Lyep20	F: ACCATCGTCACAGATCGAA[a]	(ATCT)^8	217–270	24	3	0.042[b]	0.119	0.780	td60
	R: CAGATCCGCTTCTTGCAAACT								
Lyep22	F: GCCCACAATTCACACGGATT	(ATC)^18	215–273	24	9	0.708	0.803	0.061	td60
	R: GGGATCGGTCATCTTGGTG[a]								
Lyep27	F: GTCCCTACTGAACAGGGCTT	(ATGT)^7	284–296	24	5	0.375	0.382	0.410	td65
	R: TTGAATTGTCATCGGCCCTT[a]								
Lyep31	F: TCGTCGAGAGTGCACACATA[a]	(ATCT)^14	280–392	21	9	0.857	0.831	0.050	td60
	R: CATTCCTGTGCTTAGACGGC								
Lyep33	F: TCTTTGCCTTCAAGTCTGGC[a]	(ACCT)^7…(ATCT)^13	303–353	23	7	0.435	0.524	0.250	td60
	R: AGTGGCGGGCTATAGAAACA								

The number of individuals genotyped is *N*; size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus

[a] Indicates CAG tag (5′-CAGTCGGGCGTCATCA-3′) label

[b] Indicates significant deviations from Hardy-Weinberg expectations after Bonferroni corrections

comparisons, two loci showed significant deviations from expectations under HWE and no linkage disequilibrium was detected for any of the 66 paired loci comparisons. These microsatellite loci will allow researchers to assess the genetic variability within and between populations. Specifically, we plan to use these markers to examine the population genetics of a butterfly tied to a very specific patchy habitat.

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