

PRIMER NOTE

Isolation and characterization of microsatellite loci from the Blanding's turtle, *Emydoidea blandingii*

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Abstract

Independent microsatellite development efforts at the University of Miami and Dalhousie University resulted in seven microsatellite loci for the Blanding's turtle (*Emydoidea blandingii*). The level of polymorphism ranged from three to 15 alleles with observed heterozygosities ranging from 0.31 to 0.95. Cross species amplification suggests that these loci may have utility in other turtle species.

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Blanding's turtle, *Emydoidea blandingii*, has received increasing attention over the past 30 years, as concern for its conservation has grown. The species' range centres on the Great Lakes region of North America, but discontinuous populations occur from west-central Nebraska to Nova Scotia. The combination of small population size and extended terrestrial nesting movement (Congdon *et al.* 1983; Ross & Anderson 1990) has left the species vulnerable to habitat loss, alteration and fragmentation (Kiviat 1997). Because molecular tools are potentially valuable to conservation and management (but see Steinberg & Jordan 1999 for a critical assessment), we have developed microsatellite markers for *Emydoidea* to examine paternity, male reproductive success, population genetic structure and phylogeography.

Microsatellite markers were developed independently at the University of Miami (FL) and Dalhousie University. At Miami, a partial genomic library was created in λ ZAP Express (Stratagene, La Jolla, California, USA) following methodology outlined by Hughes & Moralez DeLoach (1997). Approximately 180 000 clones were screened separately with $\alpha^{32}\text{P}$ -labelled AAT₍₁₀₎ and GT₍₁₅₎ oligonucleotides. A total of 60 AAT₍₁₀₎ and 15 GT₍₁₅₎ positive clones were isolated and sequenced with an ABI 310 genetic

analyser using Big Dye™ Terminator Cycle Sequencing chemistry (PE Applied Biosystems, Foster City CA). Primers were designed using OLIGO 4.0 software (National Biosciences Inc., Plymouth, MN, USA) for 11 clones containing eight or more uninterrupted tandem AAT repeats, and for two clones containing 12 or more GT repeats.

At Dalhousie University, 65 μg of *E. blandingii* DNA was digested with *Sau3a*. Digested DNA was separated in a 1% agarose gel, the gel containing fragments from 300 to 700 base pairs (bp) was removed, and the fragments were recovered by phenol extraction. The resultant purified fragments were ligated into pUC18 and transformed into *E. coli* DH5 α (Hughes & Queller 1993). A second library used a slightly different initial approach: 80 μg of *E. blandingii* DNA was digested with a combination of *HaeIII*, *RsaI* and *AluI*, then size-selected, ligated, and transformed as described above. Replicate lifts of both libraries were made (Hybond N+ membranes, Amersham Pharmacia Biotech, Quebec) and probed with a $\alpha^{32}\text{P}$ -labelled GT₍₁₅₎ oligonucleotide probe. Of 66 positive colonies sequenced via T7 DNA Polymerase Sequencing Kit (USB) using nucleotides labelled with ³⁵S, 16 contained GT tandem repeats, but only 10 clones contained sequence suitable for primer design.

An additional locus was modified from a locus developed for sea turtles (FitzSimmons *et al.* 1995). The locus Cm84 was polymorphic, but yielded products greater than 350 bp. For nonautomated methods the large product

Table 1 Characteristics of polymorphic microsatellite loci developed for the Blanding's turtle, *Emydoidea blandingii*

| Primer | T_a (°C) | Repeat motif | Allele | Alleles Size (bps) | H_E | H_O | primer sequence (5' → 3') |
|--------|------------|--------------|--------|--------------------|-------|-------|--|
| Eb 05† | 60 | AAT | 5 | 45–57 | 0.64 | 0.61 | F- GCC AGG AAC AAT GTT TTAS R- TTG GCA TTC TAC ACA TAA TAA |
| Eb 09* | 55 | CA | 15 | 128–160 | 0.83 | 0.75 | F- TTG AAT TAG CTC ATA AGC AC R- TCA TAA TGT GAA TTG GTC TC |
| Eb 11* | 55 | CA | 13 | 172–204 | 0.79 | 0.71 | F- GAG GAT CAG AAT GTT CAG AC R- TCT GAC TTG AAT TAA ACC TC |
| Eb 12† | 55 | CA | 7 | 119–141 | 0.34 | 0.31 | F- GTC CCT AGA TTT AAC TGA TAA ACT TG§ R- AGG GTG GAG GAA GAG GAA TAG |
| Eb 15* | 44 | CA | 34 | 147–186 | 0.95 | 0.92 | F- AAT TGA TCC CTT GAT CCG R- TCA GGA CTA TGA GGA AGC |
| Eb 17† | 55 | AAT | 5 | 94–109 | 0.69 | 0.69 | F- CCC ACA AAA GTA GAC ACC TAT§ R- GGC ACT GAA ATA AGA GAA AGT A |
| Eb 19† | 55 | AAT | 3 | 100–109 | 0.58 | 0.61 | F- AGG GCT CTG AAG CAC TAA AGT AAS R- CTC CGG CTT TTC ATT TGT GT |

T_a , annealing temperature, †, observed heterozygosity derived from Michigan samples only ($N = 175$), *, observed heterozygosity derived from range-wide sample ($N = 100$), §, dye labelled primer (for specific dye see text).

size was difficult to score reliably. The polymerase chain reaction (PCR) product was cloned, sequenced, and new primers designed to produce products approximately 160 bp in length. This redesigned primer set was designated Eb 15. An additional locus (Cc 7) developed by FitzSimmons *et al.* (1995) was also polymorphic in *Emydoidea* (7 alleles, $H_O = 0.644$).

Amplification was carried out in 10 μ L total volume containing ≈ 10 –50 ng of genomic DNA, 125 μ M dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.1% triton 100, 500 nm of each primer, and 0.25 U *Taq* DNA polymerase (Perkin-Elmer). After an initial 5 m at 95 °C, reactions were subjected to 30 cycles of 30 s at 92 °C, followed by 30 s at the annealing temperature (T_a) (Table 1), and 30 s at 72 °C. Reactions were cycled using the 'tube control' function of a Hybaid thermocycler. The PCR products were initially evaluated for amplification and polymorphism using non-denaturing 4% PAGE and visualized using ethidium bromide staining (Sambrook *et al.* 1989). For the Miami group, 3 of 11 AAT (Eb 05, Eb 17, Eb 19) and 1 of 2 GT (Eb 12) containing loci were polymorphic (Table 1). While all 10 loci designed at Dalhousie successfully amplified, only Eb 09 and Eb 11 contained more than two alleles.

Two independent population samples were used to assess the degree of polymorphism of loci developed at Miami and Dalhousie Universities, respectively. The four Miami loci (Eb 05, Eb 12, Eb 17, Eb 19), were screened on 175 individuals from the University of Michigan's E.S. George Reserve (Pinckney, MI, USA; 84°00' long., 42°27' lat.) and 6 individuals from Illinois (Dupage County, IL, USA). Primer pairs for the five polymorphic Miami loci were labelled with one of following fluorescent dyes: HEX (Eb 19); TET (Eb 05, Eb 17); or FAM (Eb 12). Except for Eb

17, whose PCR product was diluted 4 \times prior to use, 1 μ L of PCR product was combined with 12 μ L deionized formamide and 0.5 μ L GENESCAN™ 500 bp TAMRA size standard (ABI Prism, Applied Biosystems, Foster City CA), then denatured and electrophoresed on an ABI 310 genetic analyser. Microsatellites were sized and visualized using GENESCAN™ analysis software v.3.1 (ABI Prism) and GENOTYPER™ v2.1 software. The Dalhousie loci (Eb 09, Eb 11, Eb 15) were evaluated on 100 animals collected from across the species range. One primer at each locus was end-labelled with ^{33}P , and PCR products were electrophoresed on 8% polyacrylamide gels and exposed using Kodak X-Omat film. Allele size was determined by comparison to M13 sequence. The number of alleles per each locus, allele size range, and observed (H_O) and expected (H_E) heterozygosity are shown in Table 1.

To assess the interspecific utility of the *Emydoidea* primers, we attempted to amplify the primer sets in *Chelydra serpentina*, *Chyrsemys picta*, *Gopherus polyphemus*, and *Trionyx spiniferus*. Annealing was assessed at three temperatures (50 °C, 55 °C, 60 °C) using a constant 1.5 mM $MgCl_2$. Although polymorphism was not assessed, developed primers did produce products in all four species examined (Table 2).

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Table 2 Cross-species amplification of microsatellite loci isolated from Blanding's turtle, *Emydoidea blandingii*

| Species | <i>n</i> | Eb 05 | Eb 09 | Eb 11 | Eb 12 | Eb 15 | Eb 17 | Eb 19 |
|----------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| <i>Chelydra serpentina</i> | 3 | + | – | – | + | + | + | + |
| <i>Chyrsemys picta</i> | 4 | – | – | – | + | + | + | + |
| <i>Gopherus polyphemus</i> | 2 | + | + | – | – | + | + | – |
| <i>Trionyx spiniferus</i> | 4 | – | – | – | – | – | + | + |
| Accession number | | AF416293 | AF411049 | AF411050 | AF416294 | AF411051 | AF416295 | AF416296 |

+, successful amplification within the expected size range; –, smear or no amplification.

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